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Abstract.

Pulmonary dysfunction in patients with the adult respiratory distress syndrome (ARDS) is nearly always associated with damage to other organs, although such damage may not be clinically apparent. When non-pulmonary in origin ARDS should be considered as an element of multi system organ failure (MSOF). Most patients with MSOF do not die from hypoxemia. Major trauma is nearly always associated with some pulmonary dysfunction, although often subclinical. Medical conditions such as septicaemia, trauma, pancreatitis or burns cause severe inflammatory cascade activation (SICA), which is the most likely cause of MSOF.

Oxygen metabolism is central to the development of SICA. Once established SICA causes redistribution of blood flow to and within vital organs. Organs needing a high oxygen delivery (e.g. heart or brain) are deprived of blood by the relatively high flow in less dependant organs (e.g. skin, kidney, or splanchnic bed). In this condition, blood flow is redistributed, producing a relative ischemia and reduction in the production of ATP. This ischemia promotes the formation of super oxides by xanthine oxidase, causing further tissue damage.

Ultrastructure studies show that leucocytes aggregate in the lungs, liver and spleen and tissue damage is greatest in these organs. But, even when there is little leucocyte aggregation in the lungs, considerable tissue damage is observed. Leucocytes can be induced to aggregate in the lungs without causing tissue damage. Leucocytes do not aggregate in significant numbers in the heart, kidneys or muscle. Leucocytes are therefore important in the inflammatory cascade of MSOF but not essential.

Although there are many biochemical mediators of MSOF, the choke point enzyme phospholipase is the most important and its inhibition may be important in treating the condition.

Chapter 1.

Introduction.

Single organ failure such as myocardial infarction, with cardiogenic shock or renal failure has long been recognised as a cause of mortality. But with the establishment of intensive therapy units (ITU) in the late 1960's, a new form of organ failure, involving the lungs, unrelated to the original injury, was identified. This condition is referred to as the adult respiratory distress syndrome (ARDS). The syndrome originates from a wide variety of diffuse injuries, which either directly or indirectly cause damage to the lung parenchyma.

In 1967, Ashbaugh et al. first described ARDS. Patients had pulmonary oedema, lung failure and arterial blood hypoxemia in response to non-pulmonary injuries. By the mid to late 1970's it was understood that ARDS was also associated with the sequential failure of liver, kidney, brain, blood gastro-intestinal tract, vascular tone and heart, and was usually followed by death (Tilney et al. 1973, Pratt. 1979). This syndrome of sequential multi-system organ failure (MSOF) is now well described. Current intensive therapy unit (ITU) technology allows the support of patients with single and multiple organ failures who would previously have failed to survive the initial insult or its immediate aftermath. Unfortunately these patients still die at a similar rate as in the pre-ITU period.

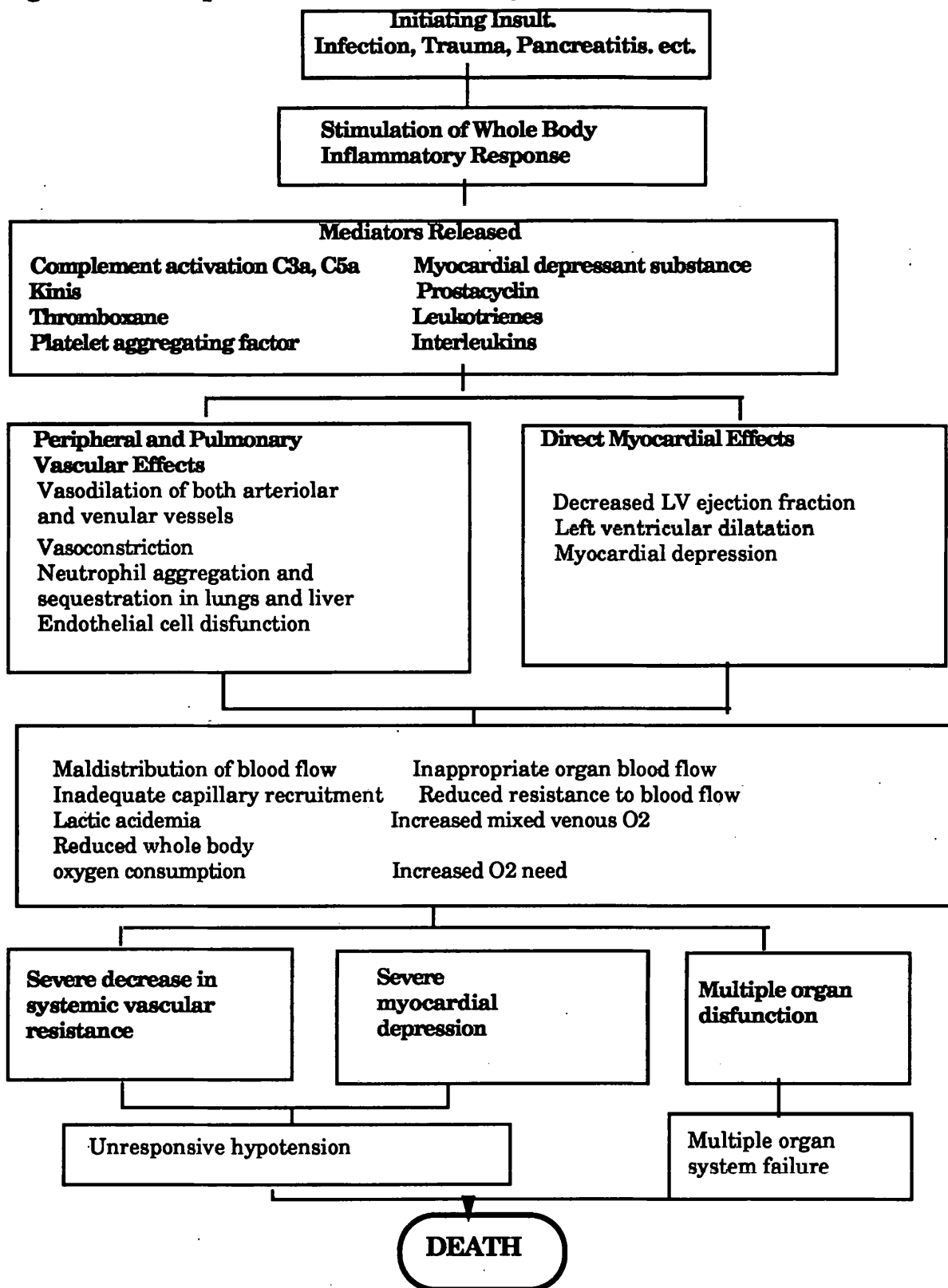
Diagnosis and symptoms.

The first manifestation of the complex medical syndrome ARDS, is the occurrence of breathlessness, in patients who often do not have any direct pulmonary injury. They develop hypoxemia and cyanosis, that is refractory not only to nasal oxygen, but also to intermittent positive pressure mechanical ventilation. These patients have to be mechanically ventilated to maintain adequate arterial blood oxygenation. The partial pressure of arterial blood oxygen should be roughly equivalent to that of atmospheric

oxygen. But in ARDS patients, the difference between inspired and arterial oxygen tension is markedly increased; often 200 to 500 mmHg, representing a large shift in the pressure saturation graph from left to right. The chest X-ray shows bilateral shadowing over most of the lungs, thought to be due to oedema (Ashbaugh et al. 1967), and the heart has a normal cardiac silhouette. This pulmonary oedema develops in the absence of any heart failure. When pulmonary oedema occurs without heart failure it is most likely due to an increase in the permeability of pulmonary capillaries. This so called "low pressure oedema" is typical of the syndrome. The permeability change was originally thought to be due to the absence of alveolar surfactant, similar to the condition, respiratory distress syndrome, seen in small children. Consequently it was called the "adult respiratory distress syndrome" (ARDS) by Ashbaugh et al. (1967).

Typical clinical patterns of patients who develop MSOF in the ITU have been established and are reported in such work as that of Cerra (1987) and Siegel and Cerra (1979). The development of MSOF is shown in Figure 1. This flow diagram shows that after a major insult, protein and lipid mediators are released, there is a massive whole body inflammatory response and there are pulmonary, peripheral and myocardial responses. Shock is seen to develop and there is a severe decrease in systemic vascular resistance. Severe myocardial depression occurs and dysfunction develops in several organs. If unresponsive hypotension and MSOF occurs, then the patient dies.

Figure 1. Development of multi ststem organ failure.



When many of these patients are admitted to the ITU, they already have shock, with low cardiac output. The fall in cardiac output (CO) in the majority of cases indicates a fall in venous return due to hypovolaemia. The cardiac output can be rapidly increased by blood volume expansion. The effect of fluid loading on left atrial pressure often indicates the heart has a limited reserve and will not function normally. The CO is in fact lower than would be expected for a given left atrial pressure, indicating myocardial depression. There is little or no rise of CO when volume expansion therapy is associated with a rapid rise in left atrial pressure. When the patient is resuscitated and the initiating event controlled, the patients often appears to do well. Several days later there is an insidious onset of low grade fever, increased heart rate and breathlessness with the appearance of diffuse patchy infiltrates of both lungs on the chest X-ray. The patient has some alteration in mental status, with normal renal and hepatic function based on standard laboratory tests. The breathlessness continues, and intubation and ventilator assistance, usually involving positive end expiratory pressure is required. The elasticity of the lungs decreases, and its capacity to oxygenate blood declines.

The patient usually stabilises haemodynamically and has a relatively high urine output and cardiac output (CO). Hyperglycemia, low systemic vascular resistance (SVR), lactic acidosis, and raised oxygen consumption (VO_2) are often seen.

The patient subsequently shows evidence of disseminated intravascular coagulation and renal and hepatic failure develop. The hyperdynamic and hypermetabolic states become more pronounced with further increases in blood sugar and lactate; there is a further increase in VO_2 and CO with a fall in SVR. There are reduced levels of hepatic proteins such as albumin, transferrin, prealbumin, and retinol-binding protein. There is a progressive need for drugs to maintain blood pressure and CO, and fluid to support blood volume and cardiac preload. The patients can

survive about 21-28 days, providing they can maintain a high CO, if they are unable to sustain such a CO, then death occurs much more rapidly.

Prognosis.

The transition to MSOF is a significant prognostic event. It marks the change in mortality from the 25%-40% to 40%-60% range in the early stage and 90%-100% in the late stage. The differentiation between early and late MSOF is primarily the degree of liver and renal failure.

Mechanism.

Scientific interest in ARDS developed because the pragmatic medical approach of treating the symptoms of the condition proved to be inadequate. A thorough understanding of the disease mechanisms is required to initiate adequate patient therapy. Scientific progress in understanding the condition rests heavily on the development of animal models showing symptoms that resemble ARDS. None of these animal studies truly reflects the condition. They are used to investigate specific features of the condition and can only have limited value. Extrapolation to the clinical condition requires considerable caution. But these studies have allowed investigation of organ ultrastructure and the development of the theory of whole body inflammation.

Initially, medical treatment for ARDS was empirical, it involved treating the symptoms as they arose. The last ten years have witnessed progress in strategies for support of patients with ARDS. These strategies are nutritional (Driver and LeBrun 1980), application of positive airway pressure and the use of high technology ventilators (Petty 1982), measurement and manipulation of intravascular pressures, blood volume and flow rates (Haynes 1982).

Unfortunately the 40-80% mortality from ARDS is virtually unchanged from the earliest reports (Petty 1975, Fowler et al. 1982). Those

individuals most at risk are often young, previously healthy people, who may have been expected to lead productive lives if they had survived ARDS. The implication is that we have not yet made the first small step in preventing or reversing the pathogenic mechanisms of ARDS. It has become clear that improved care will not improve the overall mortality from this disorder. Progress must come from undiscovered or unapplied basic insights of scientific understanding as the disease mechanism becomes better understood.

Numerous primary pathological conditions causing lung damage and arterial blood hypoxemia, but not initially involving the lungs have been known for many years. Lung damage was initially thought to be specific to the initiating illness and it was not until 1967 that the lung damage was recognised to be the same, regardless of the initiating illness. This was not altogether surprising as the lungs have a limited number of responses to the different insults that they sustain. These insults can be air-borne, direct injury or blood-borne. The extra-pulmonary insults are varied and include sepsis, trauma, shock, burns or pancreatitis. But the end stage of all types of acute respiratory insufficiency are remarkably similar in their morphological, functional and clinical features.

In some patients, control of the initiating event and supportive measures can lead to recovery. But several clinical settings are associated with the transition from the hypermetabolic state to organ failure. These include a persistent unrecognised perfusion deficit; a persistent focus of infection; or a persistent focus of inflammation such as pancreatitis or a leg fracture that has not been debrided and internally fixed within 24 hours.

An analysis of the development of ARDS over time in such patients gives an illuminating picture. First is the inciting event coupled with the host response. An inciting event can be assigned in about 80% of cases of ARDS (Fowler et al. 1982, Pepe et al. 1982), into one of nine categories:

- i. sepsis,

- ii. multiple trauma,
- iii. aspiration of gastric contents or near drowning,
- iv. multiple blood transfusions,
- v. prolonged hypotension,
- vi. burns,
- vii. pancreatitis,
- viii. focal pneumonia requiring intensive care,
- ix. lung contusion.

In each of these categories mentioned a minority of patients develop ARDS (1-30%) raising the question of risk characteristics in the host for the development of the condition. It is also unknown why some patients survive this condition and others progress to irreversible ARDS and MSOF. Equally important is the question of the common features of these nine categories allowing the development of ARDS. Are there pathogenic mechanisms common to these events?

Pulmonary oedema is a particularly important feature of ARDS. The oedema is not due to cardiac failure, as these patients usually have normal left atrial pressure. It was initially thought to be due to massive fluid overload during shock resuscitation with altered capillary hydrostatic and protein osmotic pressures. This oedema is now thought to be due to increased permeability of the pulmonary vascular bed. By the Starling equation (Starling 1896) transduction of fluid across the vascular bed into the interstitium depends on two opposing forces, hydrostatic pressure and oncotic pressure, and on the integrity or permeability of the filtering membrane. In conditions of increased permeability, more fluid will cross the membrane for any given net driving force, and the fluid will have a higher protein concentration. Fluid obtained from the airways of patients with ARDS has a higher protein concentration than from normals or from patients with cardiogenic pulmonary oedema (Newman 1985).

This increased membrane permeability has led to the assumption that the inflammatory process is involved in the pathophysiology of ARDS. Neutrophils have been implicated as a major mediator of injury because of their presence in the lungs of patients with ARDS (Schnells et al. 1980) and animal models of the condition (Tighe et al. 1989). Neutrophils have been found in increased numbers in the lungs of patients dying of ARDS (Ratliff et al. 1971) and radio labelled neutrophils have been shown by lung scan to sequester in the lungs of these patients. In animal models of this condition, the peripheral blood leucocyte count decreases to below 50% of baseline and pulmonary leucostasis is seen. The degree of hypoxemia has been shown to increase as the peripheral leukocyte count decreases (Snapper et al. 1983). In sheep made leucopaenic by nitrogen mustard there was an attenuation of pulmonary oedema and capillary permeability (Burford and Burbank 1945).

The mechanism by which neutrophils are activated to take part in this syndrome has been thought to be by the action of the complement fragment C5a. Neutrophil induced injury is thought to be associated with the production of highly toxic oxygen radical molecules. The free radical superoxide can participate in several chemical reactions yielding hydrogen peroxide and the hydroxyl radical (OH^\cdot). These potent oxidising compounds can cause injury to a number of enzyme systems and can attack cell membranes.

The lungs of patients who die from ARDS reveal variable degrees of inflammation and fibrosis, with damage to cells both on the vascular and airway side of the alveolae. The alveolar are often collapsed and contain hyaline membranes. They are typically airless and will sink when immersed in water, having a similar appearance to liver when cut. Ultrastructure studies show leucostasis with capillary endothelial and alveolar epithelial damage.

The inflammatory cascade involving the production of lipxygenases and cyclo-oxygenases from arachidonic acid by the action of phospholipases

is thought to be partly responsible for the systemic changes associated with ARDS. Thromboxane, prostacyclin, platelet activating factor and leukotrienes are the individual compounds thought to be involved with the local inflammatory response in ARDS.

Tissue ischaemia could be responsible for organic damage and the consequent initiation of the inflammatory cascade. During tissue injury from what ever cause there is a consequent increase in metabolic rate with an increased oxygen need. If this need is not satisfied then the resulting oxygen debt can cause tissue ischaemia to develop. Tissue ischaemia results in a dramatic fall in systemic vascular resistance (SVR), allowing the CO to increase by reducing after-load. This is the system mechanism to increase tissue oxygen consumption (VO_2) by increasing oxygen delivery (DO_2) through an increase in CO. If survival is to occur then the increase in VO_2 must be sufficient to meet the increased metabolic demands and prevent further tissue injury and inflammatory amplification.

Conclusion.

A major difficulty in understanding the condition has been the inability to appreciate that it is not only a pulmonary problem. It involves damage to other organs such as liver, kidneys, heart, pancreas and the gastro-intestinal tract. Most patients do not in fact die from hypoxemia, as had been previously thought: only 20% developed terminal hypoxemia. The name ARDS has become a major problem in attempts to elucidate the pathophysiology, as it concentrates the attention too firmly on the lungs. Pulmonary dysfunction in this condition is only the pulmonary aspect of a larger syndrome. ARDS would have been better named multi-system organ failure.

This thesis will initially describe the clinical signs of the MSOF, giving an historical review, and a description of ultrastructural changes. The importance of sepsis, trauma and oxygen metabolism, will be

considered to show their common characteristics in the development of MSOF. It will follow the development of MSOF as shown in figure 1. The inflammatory process will be considered, involving cell bodies, especially neutrophils and monocytes and their interaction with endothelium. The action of the protein mediators, complement, the kinins and the cytokines will be examined, as will the lipid mediators, the cyclo-oxygenases and the lipoxygenases.

Chapter 2.

Historical Background

Introduction

ARDS is a common cause of acute respiratory failure, called by many different names shown in Table 1, they are associated with the insult preceding the pulmonary dysfunction. They reflect the numerous unrelated conditions from which an understanding of this syndrome developed.

Table 1. Alternative names for ARDS.

Adult hyaline membrane disease
Bronchopulmonary dysplasia
Congestive atelectasis
DaNang Lung
Haemorrhagic atelectasis
Haemorrhagic lung syndrome
Hypoxic hyperventilation
Noncardiogenic pulmonary oedema
Oxygen toxicity
Post-perfusion lung
Post-transfusion lung
Post-traumatic pulmonary insufficiency
Progressive respiratory distress
Pulmonary contusion
Pulmonary microembolism
Pump lung
Respiratory insufficiency syndrome
Septic lung syndrome
Shock lung
Stiff lung syndrome
Transplant lung
Traumatic wet lung

Wet lung

White lung syndrome

Table 2, shows that acute lung injury can develop from a wide variety of causes, either directly or indirectly involving the lungs. In 1967 Ashbaugh et al. established the name "adult respiratory distress syndrome". Until that time it was thought that all these differing names reflected separate pulmonary conditions.

Table 2. Causes of ARDS.

Shock of any cause

Septic

Hypovolaemic

Infection

Pulmonary

pneumonia

Non-pulmonary sepsis

Bacterial (Gram negative or positive)

Viral

Fungal infection

Trauma

Fat embolism

Lung contusion

Non thoracic trauma

Head injury

Haemorrhagic hypotension

Abdominal surgery

Battlefield trauma

Fluid aspiration

Gastric juice

Near drowning

Hydrocarbon fluids

Drug toxicity or sensitivity

Heroin

Methadone

Propoxyphene

Barbiturates

Paraquat

Bleomycin

Inhaled toxins

Oxygen

Smoke (containing hydrogen cyanide or carbon monoxide)

Corrosive chemicals (nitrous oxide, chlorine, ammonia, phosgene, or cadmium)

Haematological disorders

Disseminated intravascular coagulation

Massive blood transfusions

Metabolic disorders

Pancreatitis

Uraemia

Others

Post cardiopulmonary by-pass

Pneumonia and ARDS.

Both pneumonia and ARDS involve severe pulmonary tissue damage and although the definition of ARDS is very specific, its association with pneumonia has lead to considerable confusion. The distinction between pneumonia and ARDS may require sophisticated monitoring and tests to distinguish between them that are not always available. Patients with

pneumonia often die from ARDS. Historically deaths from ARDS were most likely recorded as pneumonia. Records of the cause of death contain an over estimation of pneumonia cases and an under estimation of ARDS. The problem arises over the historic position of pneumonia in medical practice. It has been a feared disease with a high mortality, presenting as an infection of one or more lobes of the lung by bacteria, virus or fungus. It is an infection of the alveolae, forming a solid mass with the production of purulent sputum, but little oedema. On X-ray, unlike ARDS it may only involve several lobes, but unfortunately it may also involve both lungs when it is indistinguishable from ARDS and is referred to as broncho-pneumonia. Additionally when pneumonia involves only one lung, the infection can cause ARDS to develop in both lungs. Before ARDS was described, all deaths from hypoxemia were called pneumonia. Pneumonia can also cause MSOF, as many patients with pneumonia have renal failure and hepatic problems. Despite the use of antibiotics, the fatality rate in pneumococcal bacteraemia remains high. Austrian and Gold (1964) described 529 adults with bacteraemic pneumococcal pneumonia who despite appropriate antibiotic therapy had a mortality of 25%. Fruchtman et al. in 1983 described 10 patients with pneumococcal pneumonia who developed ARDS. Elderly patients who died from pneumococcal pneumonia usually did so without developing ARDS. But 10 young patients, with a median age of 33 years, developed the syndrome and 5 died. The most likely cause of death among this group of patients was the development of ARDS and MSOF as a complication of pneumonia.

The development of ARDS and MSOF is likely to arise from the inflammatory focus provided by the tissue damage resulting from lung infection. The damaged pulmonary tissue cannot be removed or drained and therefore the cascade of events resulting in septic shock and death cannot be stopped. The use of antibiotics, when given early, could stop the spread of the disease and by containing the damage to pulmonary tissue,

prevent the development of a large persistent focus which initiates the inflammatory response. But, when ARDS is established they are unlikely to reverse the course of the syndrome.

Military multi- trauma.

The history of ARDS has been seen more in connection with trauma especially when associated with the battlefield. When a soldier dies from pulmonary dysfunction several days after successful surgical repair of leg trauma the problem is quite apparent. Unfortunately battlefield trauma was often associated with infection, whether real or assumed, and was thought to be the cause of death. Only since the end of the second world war has the distinction between infection and trauma been easily and clearly made.

Pulmonary damage in response to battlefield injuries has been known for many years. Descriptions of the condition in response to trauma without sepsis during World War 1 are very uncommon. Mayo-Robson in 1918 stated "virtually all war wounds, with the exception of some bullet wounds, are not only septic but virulently septic; this is due to contamination of the skin and clothing, as well as the fragments of shell and other foreign bodies driven into the tissues, by the germ saturated soil of the highly cultivated lands of France and Belgium."

Pasteur (1913) is often quoted in current literature as describing ARDS in wounded soldiers during the first world war. He is supposed to have described pulmonary dysfunction as "acute massive collapse," in response to battlefield injuries. His work was in fact produced in 1913, a year before the outbreak of the war, and was concerned with pulmonary problems occurring after abdominal surgery. The "acute massive collapse," he described was rarely fatal and did not resemble ARDS. Most patients developed this condition after surgery but recovered from it.

Soldiers of World War 1 did not receive fluid replacement therapy and arrived at hospital for surgical treatment at least 72 hours after injury.

Thus most of the severely injured patients who may have developed MSOF, died before arrival at the hospital from their original injuries or shock. Those who did arrive at the institution would be shocked and cyanotic, their deaths recorded as due to either infection or the original trauma.

During World War II, although the wounded were hospitalised at an increased speed, Burford et al. in 1945 showed that although most patients were first seen more than 72 hours after injury, about 30% were seen within 24 hours and treated rapidly. They were shocked and cyanotic before undergoing surgery and received some fluid replacement. Unfortunately their life expectancy was only extended several days. An accurate way of measuring fluid volume was not available, and the fluid replacement therapy was usually inadequate. Consequently the patients continued to suffer from shock, hypotension, and usually died from renal failure. There was little mention of sepsis in these traumatised subjects.

Although Burford (1945) further described "traumatic wet lung" among his wounded patients, examination of his published chest X-rays show that the patients were suffering from lobular pulmonary oedema as a result of contusions of the lung. This "traumatic wet lung" is not the low pressure bi-lateral pulmonary oedema associated with ARDS, and the patient usually recovered.

Mallory et al. (1950) described pulmonary lesions in tissue taken from patients who died from shock during World War II. They originally examined tissue at necropsy from battle casualties dying up to 12 hours after injury and found little evidence of histological changes specifically associated with shock. However when they extended their investigation to 60 patients who survived injury more than 18 hours they found a constant pattern of specific pulmonary changes. The lungs were never normal and had a variety of pathological features. It can be seen in Table 3 that all lungs examined were congested, and most showed oedema, collapse and haemorrhage.

Table 3. Pulmonary pathological finding in deceased multi-traumatised soldiers who survived shock for at least 18 hours. (Mallory et al. 1950)

Symptoms	% of symptoms present
congestion	100
oedema	85
atelectasis	70
intra-alveolar haemorrhage	55
interstitial haemorrhage	25
pneumonia	30

Mallory et al. (1950) established that pulmonary oedema was never a cause of shock, but developed as a result of the shocked condition, often in association with sepsis and acute renal failure. They claimed that the oedema was not due to fluid overload as infusions of volume expanders or blood were strictly controlled, although they did not report how the latter was achieved. It is likely that these patients were shocked due to this fluid restriction. The importance of the work of Mallory et al (1950) is that it established for the first time the relationship between shock in traumatised patients and the development of lung damage. Secondly that it only occurred in those patients who survived the initial injury for at least 18 hours. Death earlier than this period was usually due to the original injury. They additionally found no correlation between fat embolism, shock and pulmonary dysfunction.

Jenkins et al. in 1950 were the first group to understand that they were dealing with an unfamiliar pulmonary condition. They named the condition "congestive atelectasis". They described the clinical course of 8 patients with "congestive atelectasis" and presented the autopsy reports of the 7 who died. All the patients had abdominal surgery for a variety of reasons from gunshot wounds and ruptured appendix to perforated ulcers. On x-ray they could see that some of the patients had pulmonary oedema,

thought to be associated with left heart failure. Although, they gave 7 of their patients digitalis, none showed any improvement and all died. From these observations they deduced that the oedema was unlikely to be cardiogenic in origin, as some patients should have improved with treatment. They also noted that, unlike obstructive atelectasis described by Pasteur in 1913, the congestive type always effected both lungs and was nearly always fatal. They referred to autopsy reports showing that 6 patients having abdominal surgery for gunshot wounds or gastric problems had evidence of non-cardiac pulmonary oedema and capillary congestion, symptomatic of ARDS.

They also performed some studies on dogs to elucidate the aetiology of the condition. Blood was radio-labelled with phosphorous 32 and a plexiglas window was inserted in the chest to view the lungs. When a leg tourniquet, previously fastened for 90 minutes was released, there was a threefold increase in pulmonary blood volume and haemorrhagic patches developed. The lungs became very stiff showing a loss of compliance when the left atrial pressure fell. The opposite would have been expected if the oedema was due to heart failure. They had shown, for the first time, the presence of low pressure permeability oedema in the lungs. On autopsy, lung tissue appeared similar to liver, it was congested and oedematous. In their radio-labelling studies, they observed a threefold increase in emissions from the lungs. They thought it represented an increase in pulmonary blood volume as they considered that P^{32} only labelled red blood cells. It undoubtedly also labelled leucocytes, and most likely represented the discovery of the now well known pulmonary leucostasis occurring in ARDS.

Unfortunately, in 1950 they did not understand the findings described in their work from either the patients or animal experiments. In the patients they thought that the oedema and congestion was caused by the over administration of fluids. This thinking confused the understanding of the syndrome and lead to a fruitless argument about the relative values of

giving crystalloids or colloids to patients for many years. It also reflected the poor understanding of adequate volume therapy in these shocked patients. This attitude was to be maintained until relatively recent times.

It is interesting to note the rudimentary equipment Jenkins et al. were working with in 1948. Ventilators were not in use at that time. Pressure measurement of the left atrium involved opening the thorax of his experimental animals to insert a left atrial catheter. This latter procedure demonstrates the difficulty of measuring patients left heart pressures and consequently the realisation that low pressure oedema even existed.

Interestingly, there were no reports documenting awareness of respiratory failure after trauma or shock during the Korean war in the early 1950's.

The Vietnam war

The Vietnam war in the 1960's brought about a complete change in the treatment of multi-traumatised soldiers. They were given vigorous field resuscitation, rapid evacuation to a hospital and prompt surgical treatment of their injuries. The improved intra-vascular volume expanders, renal dialysis, ventilators and early surgery prolonged the lives of seriously wounded personnel. The period of hypovolaemic shock suffered by the soldiers was shortened and the incidence of renal failure reduced. But it was discovered that soldiers who were initially resuscitated from their battlefield injuries went on to die of severe respiratory dysfunction. Soldiers subjected to severe shock and tissue trauma were more than usually susceptible to pulmonary dysfunction and death in the post operative period.

Simmons et al. in 1969 showed that of 96 patients who died with and without thoracic wounds, only 3 had normal lung weights. Pulmonary oedema and congestion were present in 80% of cases. When they looked at 314 combat casualties suffering non-thoracic wounds, Simmons et al. found that the great majority had some degree of hypoxemia and 20% had severe

respiratory distress. Even in the absence of shock most of the wounded had respiratory dysfunction. Martin et al. in 1969 studied autopsy reports and published their findings from 100 patients. They were able to show that 62% of deaths occurred after a week, but in most patients, this was not due to the initial injury. The lung weight of 94 patients was in excess of 1000 g, suggesting considerable pulmonary congestion. Proctor et al. (1970) during the Vietnam war studied three groups of multi-traumatised soldiers: bed rest controls with minor injuries, massively traumatised without respiratory failure and a similar traumatised group with respiratory failure. They were able to show that all three groups had respiratory failure, but to different degrees. Those patients described clinically as having respiratory failure were the most severe cases, but all the patients had some degree of failure in response to their trauma. This data suggests that tissue damage, especially when associated with shock, caused the development of respiratory dysfunction, often with fatal outcome.

Civilian multi-trauma

Ashbaugh et al. (1967) were the first to describe this syndrome after trauma in civilians as a separate entity in clinical practice. They thought the condition was due the lack of alveolar surfactant as in infant respiratory distress syndrome and used the term "adult respiratory distress syndrome". This nomenclature was later shown to be erroneous, as the lack of surfactant was a result of acute lung injury not its cause. This work drew attention to the presence of a syndrome manifest by pulmonary dysfunction that is distinct from pneumonia. Many of these patients died from acute respiratory failure in the first 48-72 hours.

Petty and Ashbaugh in 1971 showed that with vigorous treatment including the use of ventilators using positive end-expiratory pressure (PEEP), fluid restriction, diuretics and salt-poor albumen, most patients

survived the early phase of respiratory distress. But unfortunately many still died at a later stage.

Ashbaugh and Petty in 1972 looked at a group of patients to determine the causes of death after they had received the therapy described in their 1971 study. They were able to show that patients rarely died from hypoxemia in the early stages, but went on to die from the failure of several other organs as well as the lungs or from sepsis. Autopsy results always showed severe lung damage, that was not necessarily the cause of death.

In this group of 51 patients, 21 survived, but 30 died. Nine of these patients did not have respiratory failure and died from other causes. Six of these 9 died from sepsis. All the remaining 21 patients had some degree of respiratory failure, and 16 had sepsis as a major cause of death. Other contributing factors were renal failure in 11, central nervous system injury in 7, disseminated intravascular coagulation in 5, cardiac failure in 4 and other causes in 8. Twenty seven of 31 patients had autopsies performed at the time of death. The lung of all these patients were heavy and airless. This important work suggests that although better therapy could prevent the patients from dying from acute respiratory failure in the first 48-72 hours, it merely allowed the development of more severe injury to the lungs and multi-organ damage to occur.

In fact MSOF was almost non-existent as a distinct syndrome before ARDS patients were ventilated. Most died from hypoxaemias before MSOF could develop as a distinct syndrome. ARDS is a clinical term expressing the most apparent feature of MSOF; hypoxemia and pulmonary X-ray pictures showing opacity. The failure of other organs such as the liver, kidney, blood and heart are not as obvious. ARDS appears to be the pulmonary aspect of the developing multi-system organ failure.

Multi system organ failure

Tilney et al. in 1973 published the first detailed study of MSOF. They reported a retrospective study in 15 patients who underwent surgery for aortic aneurysms, showing that organ failure developed in the pancreas, lungs, liver, heart, kidney, brain or gastrointestinal tract. Fourteen patients subsequently died. The pulmonary failure that developed was typical of ARDS, and the organ failure was typical of that latter recognised as MSOF. All the patients were ventilated on a mechanical respirator and the authors considered that the majority of patients died from respiratory failure. This paper was based on analysis of data extracted from the files of past patients. Although it shows the existence of MSOF it tells little of its relevance to surgery for aortic aneurysms. We do not know the occurrence of this condition in the total population of such surgical cases. We do not know why it developed or how many patients did not develop MSOF.

There was an implicit assumption that patients who die from ARDS do so from respiratory failure. They were thought to be unable to oxygenate arterial blood sufficiently, causing inadequate oxygen consumption by the body. Consequently it was assumed that if the blood could be oxygenated artificially then the patient could survive.

Pratt (1979) of the American National Heart, Lung and Blood Institute reported on a multi-centre randomised prospective study using extra-corporeal membrane oxygenation to support patients with severe ARDS between 1975-77. Half the patients were supported by oxygenator, the other half did not receive such support. They hoped to reduce mortality by giving respiratory support to these patients. But, the optimistic expectations were unfortunately not realised.

The investigators showed that membrane oxygenation had no clinical benefit, 90% of patients in both treatment groups died. It was the first major study of a large number of ARDS patients, meticulously studied before and after death. The study showed that survival could not be related to initial

lung function. Surprisingly, they found that death could be attributed to hypoxemia in only 17% of patients. Most patients with ARDS did not die from respiratory failure: They died from multi-system organ failure; renal, hepatic or cardiac or from their initial medical problem. It was also demonstrated that, although patients did not die from pulmonary changes, they showed pathological changes in up to 3 weeks that had previously been thought to take months to develop.

Important work in showing MSOF in patients previously thought to have ARDS was performed by Montgomery et al. (1985), shown in Tables 4 and 5. This work showed that most deaths from ARDS are not caused by hypoxemia.

Table 4. The outcome of 207 patients at risk from developing ARDS. (Montgomery et al. 1985)	
ARDS group	number of patients
survivors	15
deaths	32
total	47
Non-ARDS group	
survivors	105
deaths	55
total	160

They either died from their original presenting illness or injury, sepsis or the failure of other organs. They looked at 207 patients prospectively as being at risk of developing ARDS. Forty seven per cent (87 of 207) of the patients having risk factors for ARDS died. Sixty eight per cent (32 of 47) of the patients developing ARDS died, compared to 34% (55 of 160) of the patients with similar risk factors who did not develop ARDS.

Patients who died were classified into early, up to 72 hours, and late, after 72 hours from the time of their injury, are shown in Table 5. Early deaths were usually caused by the original presenting illness or injury, 8 out of 10 in the ARDS group and 24 of 25 in the non ARDS group.

Table 5. The causes of deaths in the ARDS and non-ARDS groups.(Montgomery et al. 1985)				
	ARDS group		Non-ARDS group	
	early death	late death	early death	late death
	n=10	n=22	n=25	n=30
organ failure as a cause of death				
sepsis	3	8	3	15
cardiac	1	5	1	1
respiratory	1	4	0	2
brain	3	4	13	10
haemorrhagic	2	1	8	1
hepatic	0	0	0	1

The cause of late death in both groups was from complications that developed after entry into the study, 19 of 22 in the ARDS group and 20 of 30 in the non-ARDS group. Sepsis syndrome was the primary cause of late deaths of 8 patients in the ARDS group (36%), and was a contributing factor in a further 8 patients. Cardiac dysfunction (23%) was the next biggest cause of death. But surprisingly, irreversible respiratory failure was responsible for only 5 of the 32 ARDS deaths (16%), 4 of these 5 patients had sepsis syndrome at the time of death. Most of the patients with ARDS at the time of death had multi-system organ failure and sepsis syndrome.

Montgomery et al. (1985) concluded from this study that patients with ARDS do not usually die from respiratory failure. Some died early from the underlying illness or injury, but most late deaths, with and without ARDS,

were a result of the sepsis syndrome. Most ARDS patients who died, had the failure of three or more organs.

Monitoring

The importance of monitoring, in the diagnosis, understanding and treatment of ARDS, cannot be overstated. From the early days of this century up until the mid 1950's, a suitable method of measuring a patient's blood volume, left atrial pressure or cardiac output was unavailable. Measurement of the central venous pressure (CVP), left atrial pressure (LAP) or reflected LAP, the pulmonary capillary wedge pressure (PCWP) indicates a patients' blood volume and allows control of fluid replacement therapy. Fluid replacement therapy did not start until early in the fifth decade, and did not become common place until the late 50's. Pulmonary oedema caused by (left ventricular) heart failure is diagnosed by the presence of high LAP or PCWP. When associated with a low LAP, it signifies an increased pulmonary capillary permeability. Cardiac output (CO) is a measurement of the blood volume ejected by the heart in 1 minute and is important in determining the patients haemodynamic status. According to Starlings' law (Starling 1896, 1918), CO increases with the raising of left atrial pressure. Measurement of CVP, by inserting a catheter into the right atrium via the internal jugular vein started in the mid 1950's, but was not in widespread use until the early 1960's. Although CO and left atrial pressures could be measured in the 1960's they were cumbersome techniques, not appropriate for bedside use with very sick patients.

Swan et al. in 1970 developed the use of a triple lumen balloon catheter tipped with a thermistor, easily floated into the pulmonary artery via the femoral or brachial vein, allowing blood sampling, the measurement of PCWP, pulmonary artery pressure and CO. When measurement of these parameters became more accessible, the presence of ARDS became more apparent.

The understanding of ARDS and MSOF during sepsis, unlike trauma, developed in the late 1960's and early 1970's with the widespread use of the Swan-Ganz catheter. The acceptance of thermodilution flow directed pulmonary artery catheters in the intensive care unit made the measurement of PCWP, CO and mixed venous oxygen saturation commonplace. Clinicians could characterise pulmonary oedema as either "permeability oedema" or "hydrostatic oedema". These values permitted the rapid calculation of the systemic and pulmonary vascular resistances, intrapulmonary shunt fraction, oxygen delivery to tissue and the oxygen consumption by the tissues.

Mechanical ventilators.

A ventilator is a machine that delivers pulses of gas to a patient at a controlled rate, volume and pressure. Although these machine have been used for nearly 80 years it was not until the Copenhagen poliomyelitis outbreak in 1951 that they gained widespread use. Polio damages the breathing mechanism, ultimately leading to the death of the patient unless artificial ventilation is applied. Teams of medical students were employed to pump bags filled with oxygen and nitrous oxide, and later air into the lungs of such patients to sustain life in those affected with bulbar paralysis. Some patients were hypoventilated and others hyperventilated on the wrong volume of air by over or under enthusiastic medical students. Two Danish anaesthetists designed and mass produced machines that delivered the correct volume of gas, at the required pressure, and desired rate.

Patient ventilation prior to 1951 was very poor, relying on spontaneous breathing. Thus when a patient developed a respiratory problem his demise would be fairly rapid. Under these circumstances it can be seen that patients who might have developed ARDS would die before the gross pulmonary changes could occur. Although ventilators were used widely in the operating theatre, it was not until the mid 60's that they began to be used

in intensive therapy units. Since the aggressive use of ventilation by Petty and Ashbaugh in 1971, few ARDS patients die purely of hypoxemia. Unfortunately, they usually go on to develop and die from multi-system organ failure.

Chapter 3.

Causes of ARDS and MSOF.

Section 1. Sepsis, Septic Shock and the Septic Syndrome.

Section 2. Trauma.

Section 3. Oxygen Deprivation.

Section 1.

Sepsis, Septic Shock and the Septic Syndrome.

Introduction.

Infection is considered the greatest risk factor from which ARDS is likely to develop. The terms in common use when discussing infection should first of all be defined. *Bacteraemia* indicates the presence of potentially pathogenic bacteria in the blood with few signs of infection. *Endotoxemia* denotes the presence of measurable amounts of endotoxin or lipopolysaccharide in the systemic circulation. *Sepsis* denotes infection due to a variety of microorganisms or their toxins, associated with fever and toxic reactions. *Sepsis syndrome* is defined as the systemic response to sepsis as shown by hypo- or hyper-thermia, tachycardia, tachypnea and 1 of the following; altered mental status, hypoxemia, lactic acidosis or oliguria. But there may not be a positive blood culture. *Septicaemia* is a clinical syndrome commonly considered to be caused by gram negative or positive bacteria. Such a condition is usually associated with fever, hyper or hypodynamic circulatory shock, oliguria, lacticacidosis and pulmonary oedema, and has a positive blood culture. *Septic shock* describes a condition of cardiovascular collapse due to septicaemia.

Numerous papers have been published showing the association between sepsis, especially gram negative bacteraemia, and ARDS. The most frequently isolated micro-organisms are *E. coli* (30-40%) *Klebsiella*, *Pseudomonas* and *Serratia*. Only 25-35% of patients with bacteraemia will

develop septicaemia (Ellner 1983). A study (Dupont and Spink 1969) of 172 children with gram-negative bacteraemia, showed that septic shock developed in only 25%, but they had a mortality rate of 98%. Over the past 20 years there has been a twenty-fold increase in the incidence of Gram-negative sepsis in the U.S.A. (Cavanagh et al, 1982). There are over 300,000 cases per year, 25% of whom develop septic shock (Ellner 1983) with 70,000 deaths.

Septic shock rarely presents itself as a community acquired disease. It is associated with hospitalised patients, especially those in intensive care units with indwelling catheters or undergoing abdominal surgery. Approximately 1% of hospital admissions in the U.S.A. are complicated by gram negative sepsis, the genito-urinary tract is the most common site of infection, followed by the gastrointestinal tract and the respiratory tract.

Clowes in 1974 showed by an analysis of 8000 consecutive admissions to the surgical departments of 2 hospitals in Boston, U.S.A. that there was an overall mortality of 2.1%. Respiratory failure alone was responsible for 58% of these deaths, rising to 75% when present with severe sepsis. Thus it was apparent that sepsis played the single greatest role in the induction of fatal pulmonary lesions.

Kaplan et al (1979) identified 20 cases of ARDS from 86 cases of Gram-negative bacteraemia, an incidence of 23%, as shown in Table 6. The mortality of patients with ARDS was 90%, and without ARDS 55% respectively. Shock (systolic blood pressure less than 90 mmHg) was present in 13 patients with ARDS and in 5 patients without ARDS.

Table 6. Comparative mortalities of ARDS and non-ARDS patients from 86 with Gram-negative infection.(Kaplan 1979)

	ARDS		Non-ARDS	
	n	%	n	%
Number of patients	20	23	66	76
Shock	13	65	5	8
Mortality	18	90	36	55

Postmortem of 7 patients diagnosed as having ARDS revealed 5 patients as having pathological findings, typical of ARDS. In the non-ARDS group all of the 12 patients examined postmortem showed either completely normal lungs or pathological findings not usually associated with ARDS.

Table 7. Influence of shock on 116 consecutive bacteraemic patients with and without ARDS. (Fein 1983)

	ARDS		Non-ARDS	
	n	%	n	%
Number of patients	21	18	95	82
Died	17	81	33	34
Survived	4	19	62	65
Shock present	21	100	12	15
Died in shock	17	81	11	12
Died without shock	0	0	21	22
Class of micro-organism found in the blood				
gram -ve	17	81	58	61
gram +ve	3	14	36	39
fungal	1	4	1	1

Fein et al. (1983) reported 116 consecutive patients with documented septicaemia. His data, shown in Table 7, found that ARDS occurred in 21

patients and of the 17 of whom died, 12 or 60% had gram negative infection. The original medical problem was usually the cause of death in non-ARDS patients who died without shock.

The work of Kaplan in 1979 and Fein in 1983 demonstrate the increased risk of developing ARDS when infection was present. There was a further increase when shock developed. Both factors developing together produce a very high risk of death. It should also be pointed out that there is an increased risk of shock developing in ARDS patients.

These two pieces of work unfortunately leave several questions unanswered. From what did the non-ARDS patients die? What was the nature of the shock both groups were suffering from, and was it the same type of shock? Did patients from either group suffer from the failure of organs other than the lungs? Did the heart and arterio-venous system respond in a similar manner in both groups?

The most recent large study involving sepsis and MSOF was the placebo data from the Veterans Administration study of steroid treatment in septic shock. This study by Bone et al. (1989) describes patients with the sepsis syndrome in a group of 191 prospectively evaluated patients. Bacteraemia was defined as the presence of one positive blood culture obtained within 48 hours of entry into the study. Shock was defined as a sustained decrease in the systolic blood pressure of at least 40mm Hg from baseline or a systolic BP of <90 mmHg after adequate volume replacement. The data presented in Tables 8 and 9 is that recorded in the first 24 hours of the study. Forty-five percent (84/191) of the patients were bacteraemic and would fill the traditional definition of sepsis. The other 107 patients were non-bacteraemic.

Table 8. Comparison of the 191 bacteraemic and non-bacteraemic patients with septic syndrome. (Bone et al. 1989)

variable	Bacteraemic	non-bacteraemic
number	84	107
systolic BP (mmHg)	103	103
diastolic BP (mmHg)	57	59
temperature °F	101.5	101.6
bicarbonate (mEq/L)	21	22

Table 8 shows that there is no significant difference between the bacteraemic and non-bacteraemic groups in the variables shown.

Table 9. Significant differences between septic shock and sepsis syndrome in the 191 patients studied. (Bone et al. 1989)

variable	sepsis syndrome alone	septic shock
number	122	69
systolic BP (mmHg)	114	83
diastolic BP (mmHg)	65	47
temperature °F	102.0	100.8
bicarbonate (mEq/L)	23	20

Shock was noted in 69 (36%), patients. Temperature, systolic and diastolic blood pressure, platelet count and bicarbonate were all significantly lower in the septic shock group compared to the sepsis syndrome alone. A comparison of Tables 8 and 9 shows that the patients cannot be divided by the presence or absence of bacteria. The division between the groups is better shown by comparing the presence or absence of shock.

The occurrence of septic shock was slightly higher in the bacteraemic than non-bacteremic patients (47% compared to 30%). But there was no significant difference between the Gram-negative and positive bacteraemias regarding the development of shock. There was also no significant

differences observed in the time to develop shock between the bacteraemic and non-bacteraemic patients.

Total mortality for all patients was 26% (49/191). Patients with sepsis syndrome died at significantly lower rate than those with septic shock. Bacteraemic and non-bacteraemic patients did not differ in their mortality rates.

Data relative to the development of ARDS in this study is available in 152 patients. ARDS developed in 38 patients, reversal occurred in 23 (63%) and 15 (37%) died. Unfortunately this study does not show in which groups the ARDS patients were found. But it does show that the sepsis syndrome does not require the presence of a positive blood culture or hypotension which is integral to the diagnosis of bacteraemia and septic shock respectively. The septic syndrome is defined as the systemic manifestation of presumed sepsis. It could equally be defined as the systemic response to tissue injury that does not require the presence of micro-organisms. When only 84 out of 191 patients with septic syndrome could demonstrate a positive culture for micro-organisms then, either the test is very poor, giving a very high false negative count, or there were no micro-organisms present. The other possibility is that the patients systemic response was to tissue damage, not the presence of micro-organisms. If this is true then ARDS must be considered to develop from the systemic response to tissue injury.

These three studies show one of the major problems in studying ARDS. There is an assumption that sepsis has the greatest risk for the development of ARDS. This is partly due to the large number of papers coming from hospital medical departments who naturally report on sepsis. There are no studies in the literature describing a prospective analysis of a large number of consecutive patients entering a hospital developing ARDS and describing the precipitating cause of the condition, from all the departments of a general hospital. All of the studies are selective, they are from one department of a hospital, or only include a selected group of

patients. It is necessary to perform a study including consecutive patients with positive and negative blood cultures, in addition to the other causes of ARDS.

Two aspects of the definition of sepsis are an identified source of infection and a systemic response to that infection. The systemic response includes both the hyperdynamic and hypermetabolic aspect. Those patients having a systemic response are defined as having hypermetabolism or sepsis syndrome. Only some of these patients have identifiable micro-organisms, as shown by Bone et al. (1989). Septicaemia is a systemic response to infection usually characterised by a toxic picture of fever. This infection classically produces an acute inflammatory reaction, often associated with bacteraemia. Bloodborne cytotoxic mediators appear to elicit the septic systemic response to infection and inflammation. In addition, a similar clinical picture can be evoked in catastrophic injury which is associated with tissue inflammation and necrosis, such as multiple trauma, massive burns, pancreatitis or tissue hypoxia.

Many cases of gram-negative bacteraemia originate from an obvious infectious site, e.g. peritonitis, intra-abdominal abscess, pyelonephritis or pneumonia. The majority of cultures taken from a septic patient are negative. This finding can be due the presence of antibiotics in the blood, the difficulty of finding micro-organisms in the 5 ml of blood taken for culture or the effectiveness of the reticulo-endothelial defense system in clearing the invading organisms. However, quite often and especially in cases of severely impaired host defense, no primary focus can be detected. These patients have septic syndrome. They have a systemic and metabolic response typically seen with infection, but without any apparent source. Even post mortem examination can fail to identify a septic focus.

Seidenfeld et al. described (1973) the prospective study of 129 intensive therapy unit (ITU) patients who developed ARDS, 108 of whom were infected, described in Table 10. They examined the organism responsible,

the body sites involved and the outcome of therapy. Table 10 shows the primary causes of ARDS in their patients and clearly demonstrates that infection was the principal cause. Although 73 patients had an infection as the primary cause, 108 patients developed what was thought to be an infection.

Table 10. Diagnosis associated with the development of ARDS in 129 survivors and non-survivors. (Seidenfeld et al. 1973)

	Survivors	non-survivors
	n = 37	n = 92
Cause		
Sepsis	10	36
Pneumonia	8	19
Trauma	3	6
GI bleed	2	7
Pancreatitis	3	3
Carcinoma	0	8
Post-operative	6	5
Miscellaneous	5	8

Pneumonia associated with the development of ARDS occurred in a high percentage of survivors who had it as a persistent complication (7 of 8). Only 19 non-survivors presented with pneumonia. However, 49 had it as a complication from other non-septic causes of ARDS. Tables 11 and 12 show in more detail the patients described in Table 10. Table 11 shows that the non-survival rate was far higher in patients in whom there was lung and thorax involvement.

Table 11. Clinical and autopsy diagnosis of major sites of infection in 103 patients with ARDS. (Seidenfeld et al. 1973)

	Survivors	Non-survivors
	n = 33	n = 70
Single site.		
Lung	6	45
Abdomen	10	7
Soft tissue	1	3
Other	1	4

Table 12 shows that when patients had no infection the survival rate was greater, although 33% died. But most importantly Table 12 shows when there was a positive blood culture and the site of sepsis was unknown, and

Table 12. Characteristics of infections in 129 patients with ARDS by blood culture and clinical diagnosis. (Seidenfeld et al. 1973)

	Survivors	Non-survivors
	n	n
number of patients	37	92
No infection	14	7
Positive blood culture		
site known	11	18
site unknown	0	9
Negative blood culture		
site known	8	45
Clinical sepsis	4	13
Antibiotic therapy		
Adequate	20	49
Inadequate	3	10

therefore could not be physically removed, the mortality rate was 100%. Table 12 also shows that of 108 patients thought to be infected, 53 had negative blood cultures. When the blood culture was negative, a common finding in clinical medicine, and the site unknown, the death rate was also very high. When the patients had clinical sepsis the mortality rate was 75%. The death rate was exactly the same regardless of adequate or inadequate antibiotic therapy.

These data suggest that some patients who were considered to be infected or septicaemic may never have been infected. The condition called septic shock may be a syndrome reflecting the body's response to tissue damage from many causes including bacteria. Sepsis is often associated with gram negative infection. Endotoxin is therefore thought to be the principle stimulator of this response. But gram positive infection was present in 30% of patients, consequently endotoxin was not present.

When the infection was established antibiotic therapy had little effect on mortality in this syndrome. The syndrome appears to be a devastating whole body secondary response to the damage caused by bacteria or other insults.

The importance of this work is in showing that a focus of tissue damage, whether infected or not, or containing dead bacteria, can produce the whole body inflammatory reaction that is overwhelming and causes further extensive tissue destruction.

Clinical manifestation of sepsis.

In 1888 Roux and Yersin described abnormal regulation of blood flow following an intravenous injection of live diphtheria bacilli into rabbits. Multiple haemorrhages and microthrombi were found in kidneys, lungs and adrenals. Welch and Flexner (1891) injected live diphtheria bacilli and subsequently observed thrombi occluding the lumina of the glomerular capillaries.

Mammals infected with gram-negative bacteria often develop a state of shock characterised by hypotension, disseminated intravascular coagulation, and organ dysfunction. These descriptions of the effects seen after the administration of bacteria, such as changes in blood coagulation, the occurrence of microclots and haemorrhages, are the earliest observations on the effects of bacterial products in organ dysfunction and its consequences.

In 1948 Spink et al. found that on administering antibiotics to bacteraemic patients, they became fevered, hypotensive and had tachycardia. They suggested that endotoxin from *Brucella melitensis* induced the shock like syndrome. Waisbren in 1951 further described the clinical picture of septic shock. He described two patient groups with clinically distinct characteristics. The first group was classified as toxic and consisted of animated patients with hot, dry, flushed skin, and called, "warm shock". The second group were hypotensive, lethargic and apprehensive with cold clammy skin, and referred to as "cold shock". These two groups are now recognised as the hyperdynamic and hypodynamic phases of septic shock. The term bacterial shock was not introduced until 1963 by Shubin and Weil who considered that the patients were suffering from distributive shock, so named because of the defect in blood volume distribution.

Septic Shock

The term shock (choc) was first used by the French physician LeDran in 1773 to describe the clinical sequence seen in patients after severe gunshot trauma. Clinically, shock is a syndrome that is a manifestation of circulatory failure. It is often recognised by the onset of hypotension, but does not necessarily start with it, as in septic shock. Shock is a maldistribution of the microcirculation, resulting in a reduced oxygen consumption (VO_2) relative to metabolic needs. It can be cardiogenic, which is a maldistribution

due to inadequate blood supply to the microcirculation, arising from poor cardiac function. Haemorrhagic shock is similar to cardiogenic shock but is due to inadequate blood volume circulated to the microcirculation. Both cardiogenic and haemorrhagic shock result in vasoconstriction of certain organ beds as an adaptation to maintain blood flow to vital organs such as brain and heart. But shock associated with trauma or sepsis is a distributive shock, associated with inadequate blood supply to the microcirculation, that might be considered high compared to cardiogenic or haemorrhagic shock, or even the basal state. But the blood is not distributed to organs and tissues in appropriate volumes relative to its needs. It is differentiated from the other types of shock by the reduction in systemic vascular resistance, producing vasodilatation. This dilatation is not fully compensated for by an increase in cardiac output, consequently the blood pressure falls.

The typical clinical picture of shock in septic patients is fever, confusion, shortness of breath and eventually hypoxemia and hypotension. At first the blood pressure responds to fluid administration and the patient shows evidence of a hyperdynamic circulation with an increased metabolic rate. This phase is followed by protein wasting, peripheral oedema and deteriorating function of the brain, lungs, liver and the kidney. Finally, the falling blood pressure is unresponsive to fluid administration and if cardiac stimulants (inotropes) are unable to support the circulation, the patient dies.

The initial stage of septic shock is characterised by fever, hyperventilation and respiratory alkalosis. These manifestations are associated with an increased cardiac output and pulse pressure, with a striking decline in systemic vascular resistance and narrowing of the arterio-venous oxygen difference. Oxidative metabolism is compromised and the oxygen debt is thought to be reflected by the development of lactic acidosis. This hyperdynamic state is thought to be mediated by endogenous vaso-dilators such as endorphins, kinins, prostaglandins and histamine

and is characterised by warm extremities, often referred to as "warm shock".

However, progression of the shock state is followed by the more familiar "cold shock", with progressive hypotension, cooling of the extremities and anurea. These clinical features are associated with a decreased cardiac output and pulse pressure, increased systemic vascular resistance and marked lactic acidosis. Although it should be emphasised that the concept of "cold shock" is becoming discredited, patients who develop a protracted period in this condition are more likely to be hypovolemic i.e. reduced effective blood volume. When given adequate volume resuscitation, warm shock develops. Cold shock only occurs just prior to death, due to myocardial depression and its consequent reduction in cardiac output.

Several factors may play a part in the transition from the hyperdynamic to hypodynamic septic shock. A substantial portion of the blood volume appears to be pooled in the venous capacitance vessels producing a relative hypovolaemia, such that the effective circulating blood volume is decreased. Fluid loss secondary to fever, diarrhoea, vomiting, sequestered third space fluid (interstitial) or increased capillary transduction may also contribute to the hypovolaemia and inadequate fluid administration.

Critical decreases in the venous return and consequent reduction of cardiac output produce a hypodynamic circulation. Release of endogenous catecholamines and vaso-constrictors such as vasopressin, angiotensin, 5-hydroxytryptamine, and thromboxane mediate increased sympathetic tone and systemic vascular resistance. Cardiac dysfunction secondary to myocardial ischemia or the myocardial depressant factors may also contribute to the hypodynamic state.

Although Mohr et al. (1969) showed that fluid challenge in septic shock patients failed to increase VO_2 despite haemodynamic improvement,

Kaufman et al. (1984) reported a significant increase in VO_2 with reversal of lactic acidosis, when patients were volume expanded. The volume expansion caused an increase in oxygen delivery in patients with hypovolaemic shock. Abraham et al. in 1983 showed that increases in cardiac output, DO_2 and VO_2 occurred in surviving patients with septic shock. These studies suggest that increases in oxygen consumption reflects increases in nutrient capillary blood flow. There is a greater need for oxygen as tissue repair, production of mediators and clearance of toxins is associated with an increased metabolic rate.

Metabolic changes during sepsis.

Gump et al. (1970) showed that oxygen requirements are 50% greater than normal during septic shock. The increased VO_2 in septic shock reflects the greater oxygen requirements due to hyperthermia and hypermetabolism. The hypermetabolic response is associated with hyperglycaemia, hypertriglyceridaemia and hyperuricaemia. Although sepsis is characterised by an insulin resistance, glucose oxidation is actually increased. The hyperglycaemia is primarily caused by accelerated endogenous glucose production in the liver (gluconeogenesis). Hypoglycaemia secondary to hepatic failure occurs very late in the course of sepsis. Fat is increasingly oxidised as a major fuel substrate during septic shock and lipolysis is associated with increasing blood free fatty acid levels. As metabolic failure worsens, there is decreased triglyceride clearance. Proteolysis leads to an excessive loss of body protein as amino acids are used to supply alternative energy sources. Protein catabolism is reflected by an increased ureagenesis and urinary nitrogen excretion. Progressive multiple organ failure appears to follow these catabolic derangements, most especially pulmonary and hepatic failure. Muscle protein destruction appears to be flow independent and oxygen delivery at this late stage is no longer the primary determinant of survival.

Animal models of sepsis

Goris et al. (1986) performed one of the most important studies to investigate the significance of micro-organisms in the development of ARDS and MSOF. They investigated the effects of a non-bacterial, non-endotoxic, local inflammatory stimulus on the functional and microscopic changes in distant organs on rats. They performed 3 groups of experiments, A, B. and C over a 12 day period, as shown in Table 13 and 14. Zymosan was used as it is a potent inflammatory agent, activating the complement cascade.

In experiment A, 30 male Wistar rats, (300-400g) were randomised and divided into 2 groups. Group 1 (20 rats) received an aseptic intra-peritoneal injection of zymosan, suspended in paraffin. Group 2 (10 rats) received an aseptic intra-peritoneal injection of the vehicle, paraffin.

Table 13. Time of death and positive blood cultures in experiments A and B. (Goris et al. 1986)				
positive cultures of bacteria are in brackets ()				
Time of death				
Group	n	0-3 days	9-11 days	12 days
Experiment A (normal rats)				
1zp	20	7 (7)	3 (0)	10 (1)
2p	10	0 (0)	0 (0)	10 (0)
Experiment B (germ free rats)				
1zp	10	1 (0)	0 (0)	9 (0)
2p	10	0 (0)	0 (0)	10 (0)
Experiment C (normal rats)				
control	10	0 (0)	0 (0)	10 (0)
z=zymosan, p=paraffin				

In experiment B, 20 germ free male Wistar rats, (300-400g) were kept under germ free conditions and randomly divided into 2 groups. Group 1,

received an aseptic intra-peritoneal injection of zymosan, suspended in paraffin, and group 2, received an aseptic intra-peritoneal injection of paraffin. In experiment C, 10 male Wistar rats killed after 12 hours, (300-400g) served as controls. Positive blood cultures were obtained from the peritoneal fluid. All 7 experiment A, group 1, rats that died early and also in 1 that died after 12 days were positive. Bacterial cultures of peritoneal fluid remained negative in all other rats of both group A and B. Cultures of faecal material in the germ free rats showed no bacterial growth.

The histological findings were normal in all rats from group 2 in both experiments A and B. In group 1 rats of experiments A and B, an extensive fibroblastic peritonitis was found with massive adhesions. The lungs showed extensive hyperemia with haemorrhagic spots and occasionally extensive haemorrhagic infarction.

Table 14. Relative mean organ weights of rats in experiments A, (normal) and B (germ free), groups 1 and 2. (Goris et al. 1986)			
Organ weight/body weight X100)			
Group 1 (zymosan)			
	Group A1	Group B1	Control
Lungs	1.42	0.99	0.45
Liver	4.69	4.93	3.77
Kidneys	0.84	0.94	0.66
Spleen	0.24	0.34	0.15
Group 2 (paraffin)			
	Group A2	Group B2	
Lungs	0.60	0.46	
Liver	4.14	4.11	
Kidneys	0.69	0.70	
Spleen	0.15	0.20	

Microscopically, the lungs showed interstitial and intra-alveolar oedema and greatly increased numbers of intracapillary granulocytes. The liver showed congestion and oedema and an increased number of granulocytes in the sinusoids and space of Disse. The kidney and spleen also showed signs of damage. Acute peritonitis was found in those animals that died early, but in none of the other animals.

Groups 1 and 2 were always significantly different, in experiments A and B. Although there was a qualitative similarity in histological changes in A1 and B1 there was a greater increase in organ weights in B1 compared to A1. The increase in organ weight was greater in the bacteria free animals except for the lungs.

The vital importance of this work cannot be overstated. Goris et al. (1986) have shown that bacteria free animals can develop similar organ damage to those animals with normal gut flora. Liver, kidney and spleen had a greater weight in the germ free animals than similar organs of the normal rats. The conclusion must be that organ damage results from the large area of inflammation occurring when sterile peritonitis is induced.

We (Tighe et al. 1989) examined vital organs in rabbits with peritonitis by electron microscopy and cultured the micro-organisms in those organs, our data are shown in Table 15. We demonstrated a lack of correlation between the presence of micro-organisms in an organ and tissue damage within that organ. The rabbits' hearts showed no structural changes whereas the lungs demonstrated considerable damage, but both organs produced few colonies of bacteria. The liver, spleen and kidney produced high colony counts with damage to the two former organs, but no damage to the latter. This evidence suggests that the presence of bacteria in an organ is not necessary for it to suffer damage. But their absence does not prevent damage to that organ.

Table 15. The relationship between organ damage and the presence of bacteria. (Tighe et al. 1989)

		LUNG	HEART	BLOOD	LIVER	SPLEEN	KIDNEY
	<i>Enterobacter agglomerans</i>	+	+	+	+	+	+
	<i>Strept. faecalis</i>	—	—	—	+	+	+
	<i>Bacteriodes</i>	—	—	+	+	+	+
	Gram -ve anaerobic rods	—	—	—	+	+	+
TISSUE CHANGES	Degranulating Neutrophils	+	—	—	+	+	—
	Endothelial Damage	+	—	—	+	—	—
	Fibrin Deposits	—	—	—	+	+	—
	DIC	—	—	+	—	—	—

Disseminating intravascular coagulation (DIC)

Conclusion.

ARDS and MSOF in association with generalised sepsis accounts for the death of 60% of patients dying in an ITU. As MSOF was almost non-existent before ARDS patients were ventilated mechanically, it seems that the ITU has created its own disease. There is a consensus that bacterial overgrowth in these immunologically compromised patients is the cause of this highly lethal syndrome.

But the evidence for such a causal relationship is weak:

- i). MSOF develops in patients with primarily nonbacterial problems such as severe pancreatitis or severe trauma before bacterial invasion is obvious.
- ii). Positive blood cultures are not present in many of these patients.
- iii). No single study could reliably demonstrate elevated levels of circulating endotoxin preceding MSOF.
- iv). The drainage of pus or administration of antibiotics or decontamination of the gastro-intestinal tract does not prevent or suppress MSOF.

v). A MSOF like syndrome can be induced aseptically in rats by intraperitoneal inoculation of zymosan.

Generally the therapeutic concern about the patient with sepsis has been related to choosing the proper antibiotic therapy or to deciding the optimum time for surgical drainage or excision of an organ that has become the focus of a life threatening septic process. The preoccupation is with the specific nature of the infectious agent and its progress, rather than the more general nature of the host response and support of the host defence mechanism.

The common denominator in the development of ARDS and MSOF appears to be tissue injury regardless of whether it be caused by physical trauma, lack of perfusion as in shock, or fulminating invasive sepsis.

Section 2.

Trauma

Multi-trauma had not usually been associated with ARDS and MSOF, as such patients usually died from the original injury, shock or infection. During the first world war more soldiers are thought to have died from shock and infection than from the original wound. The Vietnam war brought about an understanding of the involvement of trauma alone in causing ARDS and MSOF. Wounded soldiers were quickly airlifted to hospital, where they received immediate corrective surgery and blood replacement, were ventilated and received renal dialysis when necessary. But they still died from unexplained causes. Intensive investigations were carried out to find the reason for these deaths, although the mechanisms involved remained a mystery.

Pulmonary dysfunction in battle casualties.

A (Simmons et al. 1969) report on over 800 autopsies performed from 1966 to 1968 was published by the U.S. army in Vietnam.

Criteria for the report was;

- 1). All patients had combat wounds from missiles or blast.
- 2). None had been burned.
- 3). All were quickly evacuated to hospital, received resuscitative care, survived the initial operation and died during the post-operative period.
- 4). All patients were studied prior to the initiation of therapy.

Of 96 patients who died from wounds, after resuscitative surgery, 80% had pulmonary oedema, 60% had pulmonary congestion, and 28% had pulmonary haemorrhage. Only 1 patient had a normal lung weight and only 2 had lung weights less than 1200 g. Thoracic and non-thoracic wounds produced the same pulmonary dysfunction and was a common cause of death.

In 314 combat casualties breathing room air, an arterial pO₂ of less than 80 mmHg was found in 33% and less than 60 mmHg in 8.5% of patients. Although most patients with penetrating chest wounds had hypoxemia, 17% of 148 patients with extremities and soft tissue injuries also had hypoxemia.

The possible influence of shock was considered as a contributing factor of pulmonary dysfunction, defined as a systolic pressure of less than 60 mmHg, an arterial pH less than 7.30 or an arterial blood lactate higher than 20 mg%. Where these parameters were measured in patients with extremity and soft tissue wounds 112 patients had an arterial pO₂ of 90 or less. Although 27 had shock, surprisingly, 85 did not. Although Simmons et al. (1969) showed that there was a significant relationship between the degree of hypoxemia and the presence of shock, it was found that patients without shock still suffered pulmonary dysfunction, as seen in Table 16.

Table 16. Effect of shock on arterial oxygen tension in 148 traumatised patients. (Simmons et al. 1969)				
	shock present		shock absent	
arterial pO₂ (mmHg)	n	%	n	%
>100	7	20	29	25
80-90	16	47	72	63
60-79	7	21	10	9
40-59	2	6	3	3
<40	2	6	0	0
total	34		114	

Table 17 shows that in the absence of shock, there was a statistically significant relationship between the degree of hypoxemia and the presence of fractures in patients. In the absence of fracture or shock no patient with extremity or soft tissue injury developed hypoxemia on admission to the

hospital. These data suggest that in patients with tissue injury insufficient to cause bone fracture, hypoxemia was less likely to develop.

Table 17. Per centage of patient suffering hypoxemia (without shock) in response to fracture. (Simmons et al. 1969)		
arterial pO2 (mmHg)	Fracture (67)	No fracture (47)
>100	22	28
80-99	60	70
60-79	13	2
40-59	5	0

Table 18 shows the site of the fracture was important in the development of hypoxemia. Foot and femur fractures were associated with profound hypoxemia or shock but fibula-tibia and arm fractures were not.

Table 18. Per centage of patient suffering hypoxemia (without shock) in response to differing fracture locations. (Simmons et al. 1969)				
pO2(mmHg)	Femur (26)	Tibia-fibula (28)	Foot (9)	Arm (4)
>100	12	39	0	25
80-99	62	54	67	74
60-79	19	7	22	0
40-59	8	0	11	0

The important feature of these wounds was that they were associated with high energy trauma. All the foot injuries were caused by mines. Femur injuries were caused by high energy bullets. Tibia and fibula injuries were associated with lower energy blast and consequently less tissue contusion. Sepsis is not mentioned in this study so its absence must be assumed.

This work shows that pulmonary dysfunction is in proportion to the degree of tissue trauma suffered by the subject. When the mass of tissue

suffering contusion increased, the degree of pulmonary hypoxaemia increased.

Martin et al. (1969) studied 800 autopsy reports from the U. S. Army 9 medical laboratory in South Vietnam between 1966 and 1968 from which 100 were selected for analysis. Selection of data was made on the following criteria;

- 1). All patients had received combat wounds.
- 2). None had been burned.
- 3). All were rapidly evacuated to hospital.
- 4). The autopsy reports were sufficiently detailed with microscopic studies to permit detailed analysis.

All patients were men aged 18-47 years who had a mean survival time following injury of 6.5 days. Thirty three per cent of deaths occurred during the first 24 hours and 62% in the first week.

There was no correlation between lung weight and duration of illness or site of injury. Table 19 shows that most patients at autopsy had lungs much heavier than the normal 800 g.

Table 19. Lung weights of 100 combat casualties. (Martin et al. 1969)	
No. of patients	lung wt in g
5	<1,000
64	1,000-2,000
31	>2,000

Pulmonary oedema, congestion and alveolar hemorrhage were found in 89 of the 100 patients. The pulmonary dysfunction was rarely caused by fat embolism, only being found in 4 of the 100 patients. Septicaemia, usually associated with abdominal injury, occurred in 18 patients and was seen after the first week. These data show that the majority of patients had severe lung damage as a result of non-thoracic tissue injury. Most of these patients

died from lung failure. The majority of patients studied by Martin et al. (1969) who died of multi-trauma without infection had lungs typical of ARDS.

Proctor et al. (1970) studied 52 patients who were wounded soldiers from the U.S. Navy hospital in Da Nang, South Vietnam. They were divided into 3 groups and studied until death or survival 120 hours after injury, shown in Table 20.

Table 20. Comparison of the three groups of seriously injured patients. (Proctor et al. 1970)			
Group	1	2	3
massive abdominal and/ limb injuries.	33	0	4
minor traumatic injuries.	0	15	0
SABP < 80 mmHg	33	0	4
Respiratory problems	0	0	4
PaO ₂ < 60 mmHg	0	0	4
SABP Systolic arterial blood pressure.			

Similar massive soft tissue injuries and fractures were common to all patients in groups 1 and 3, but no patients had thoracic injuries. In group 1, were 24 patients who had limbs traumatically amputated by exploding mines. Eight patients had only abdominal injuries and 4 had both traumatic amputations and abdominal injuries. In group 2, were 15 bed rest controls who sustained only minor injuries, with no fractures or wounds requiring only local debridement. In group 3, were 4 shock lung patients with injuries similar to those of group 1. Their chest X-rays were consistent with shock lung and had clinically apparent signs of respiratory insufficiency.

Although 3 deaths occurred in group 1 they could not be attributed to pulmonary causes and at autopsy their lungs were unremarkable. Whereas

the lungs of patients in group 3, were wet, heavy and congested, typical of that seen in patients with ARDS.

Group 1 patients showed an altered pattern of respiration when compared to normal subjects. They had a decrease in tidal volume and an increase in respiration rate, resulting in very little alteration in minute volume. There was a small decrease in pulmonary compliance. Although there was an increase in resistive work per breath, there was very little change in total work per breath. When comparing normal subjects to group 1, the total work per breath was similar. But, as group 1 patients were respiring at a faster rate than normal, the total work to move a similar volume of air in group 1 patients was higher than that found in normal patients. According to the data of Proctor et al (1970) these patients were in fact working harder to move an equivalent volume of air. There was a very large increase in pulmonary A-V shunting.

This altered pattern of respiration for all the group 1 patients was compared with normal values. Tidal volume and compliance were significantly lower than normal while respiration rate, minute volume, total work per breath, total work per litre, resistive work per breath, resistive work per litre and pulmonary shunting were all significantly higher than normal values. But surprisingly, when the bed rest controls, in group 2, were compared to normal controls it was discovered that they also had a similar pattern of significant differences as in group 1 patients. Thus group 2 patients also showed abnormal pulmonary function, albeit at a better level than group 1 patients. In fact when group 1 patients were compared to group 2 patients it was seen that although they were significantly different, group 2 patients were also significantly worse than would be expected from normal subjects.

The investigation of group 1 patients characterised the respiratory pattern found in those surviving massive non-thoracic injury, haemorrhagic shock and resuscitation. Comparison of group 2 patients

with normals revealed unexpected alterations that could not be easily explained.

Comparison of the limited data collected from group 3 patients with the data from group 1, indicates that the two groups are qualitatively identical, but that alterations in group 3 are quantitatively more severe. As a result of this increased severity, the group 3 patients had clinically apparent symptoms of respiratory failure. On the basis of clinical evaluation alone, they may be set apart from group 1 patients with apparently similar injuries, and diagnosed as having shock lung. Proctor et al (1970) considered that on the basis of the measurements made, the singling out of clinically apparent cases and labelling them shock lung was not justified. Whatever the factor responsible for the alteration in respiratory function, it appeared to operate in all three groups of patients studied.

When Proctor et al (1970) calculated the oxygen consumption for group 1 patients, he found it to be high on admission, gradually decreasing to normal over the 120 hour investigation period. Proctor et al (1970) showed that a basic abnormality was an increase in oxygen requirement in the face of a ventilation perfusion mismatch, requiring increased alveolar ventilation. Because of this defect and the reduced pulmonary compliance there was an increase in the work of breathing. Proctor et al (1970) assumed that the major difference between group 1 and 3 was the inability of group 3 patients to produce enough oxygenated blood to satisfy their metabolic needs. This assumption was most likely correct but Proctor et al (1970) did not take the idea to its logical conclusion. The inadequate oxygen consumption that occurred in group 3 patients produced tissue hypoxemia and further tissue damage. If an attempt had been made to produce high oxygen delivery in these patients then their mortality may have been reduced. These data show that any tissue trauma will cause arterial blood hypoxemia. When severe, it is termed ARDS, but when less severe, producing subclinical hypoxemia it remains undiagnosed and unnamed.

It should be noted that both group 1 and group 3 were both severely injured, but group 1 were mostly traumatic amputations. Whereas group 3 patients still retained their damaged tissue. Group 1 patients had little damaged tissue to initiate a major inflammatory response, unlike group 3 patients.

Civilian trauma.

Ashbaugh et al. in 1967 described ARDS in their patients, their data are shown in Table 21. They were the first to recognise ARDS as a specific clinical entity, separate from the condition that precipitated its development.

Ashbaugh et al. (1967) looked at 12 patients with hypoxemia and loss of pulmonary compliance after a variety of insult. Seven patients had severe trauma, 4 had viral pneumonia and 1 had acute pancreatitis preceding the respiratory distress. Pulmonary dysfunction occurred from 1-96 hours after the precipitating insult. Although all 12 patients developed hypoxemia, hypotension was present in only 5 patients and acidosis ($\text{pH} < 7.34$) in 5 patients before the onset of respiratory distress.

When pulmonary compliance falls, it reflects a decrease in the elasticity of the lungs and is estimated by dividing the lung volume by the maximal breathing pressure in paralysed ventilated patients. All patients were ventilated and showed reduced compliances to between 0.009 and 0.019 l/cm of water. (normal is about 0.05-0.125 l/cm of water).

Table 21. Data from 12 patients suffering from acute respiratory distress.
(Ashbaugh et al. 1967)

illness	Hypotension	acidosis	SaO ₂
multiple trauma	++		85
multiple trauma	+++	++	72
multiple trauma	+		78
abdominal gunshot	+++	+	73
blunt chest injury	-	++	85
acute pancreatitis	+++	+	85
viral pneumonia	-		79
viral pneumonia	-		41
viral pneumonia	-	++	84
multiple trauma	-		87
viral pneumonia	-		74
chest gunshot	-		72
SaO ₂ = oxygen saturation of arterial blood			

Gross inspection of the lungs of the 7 patients who died showed them to be heavy and deep reddish-purple with an average weight of 960 g for the left and 1150 g for the right. Cut section resembled liver tissue. Microscopy revealed all major pulmonary vessels to be patent and free of thrombus. Interstitial and intra-alveolar haemorrhage and oedema were common. Alveolar macrophages were numerous and alveolar hyaline membranes were found in all but one patient.

Although they do not make such a claim, this work demonstrated the very important finding that pulmonary dysfunction occurred in the absence of shock or sepsis. While shock may be a precipitating factor for pulmonary dysfunction in some patients it was not necessary for the development of respiratory distress in the majority of patients. The correction of shock had no effect on overall mortality. Multiple trauma alone was the cause of a lethal outcome in 50% of these patients.

Hypoxemia associated with skeletal injury.

Hypoxemia associated with fractures was originally described by Sproule et al. (1964). Since that time many authors have emphasised the importance of arterial hypoxemia in relation to fat embolism. They consider that the broken bone releases fat into the circulation, subsequently becoming trapped in the lungs. In the majority of cases the hypoxemia is unrecognised clinically and is thus termed unapparent hypoxemia (as mentioned in military trauma). Typical of these studies is that of Wrobel et al. (1974), described in Table 22. They undertook a prospective analysis of 100 adults with non-thoracic trauma. The partial pressures of oxygen in arterial blood (PaO₂) were obtained on admission from patients breathing room air.

Table 22. Partial pressures of oxygen in arterial blood from 100 severely injured patients immediately after hospital admission. (Sproule et al. 1964)	
PaO₂ mmHg	Number of patients
> 100	30
80-100	30
70-80	27
< 70	13

Patients with the highest risk of hypoxemia were those with fractures of the lower extremity (femur, tibia and fibula). The majority of patients did not show clinical hypoxemia as even with low partial pressures, down to 60 mmHg, blood exhibits 98% saturation.

Although most studies of post-traumatic hypoxemia suggest it is caused by fat embolism, the diagnosis is usually based on clinical criteria, such as confusion and skin petechiae, with little attempt to show the presence of fat in the blood stream.

Hutchins and Macninol (1985) studied twenty males with one or more long bone fractures in the lower limbs. They looked for fat globules by micropore filtration and microscopic examination. They assigned patients to 2 groups and reported on the presence of fat macroglobules in the blood, as shown in Table 23.

Table 23. Association of fat in the blood with respiratory distress in 20 patients. (Hutchins and Macninol 1985)		
	profound hypoxemia	mild hypoxemia
patients	6	14
presence of blood fat	1	3

Although all the patients became hypoxemic, only six developed profound respiratory distress with significantly lower arterial oxygen tension, these patients were assigned to group 1. The other 14 patients appeared to have better pulmonary function and were assigned to group 2. Circulating fat macroglobules were identified in four cases, only one was in group 1. The other three were associated with only mild respiratory distress.

The test for fat embolism was therefore thought to be of little prognostic value. This finding casts doubt on the role of fat embolism in traumatic respiratory distress and unapparent hypoxemia. There is every likelihood that the role of fat embolism in trauma is much exaggerated. The cause of hypoxemia is more likely to be due to some lung damage, resulting from the initiation of the inflammatory process in response to tissue trauma.

Early Bone Fixation.

In Germany in 1942 Kuntscher started the procedure of internal fixation of fractured weight bearing limbs. This procedure was adopted to increase the speed of recovery and reduce the incidence of permanent

damage. In 1958, Allgower (Allgower 1977) in Switzerland, became dissatisfied with the poor results from the therapy for bone fractures. It was an unsatisfactory mixture of conservative and operative treatment, with the conservative treatment prevailing. Conservative treatment was traction for 3-4 weeks, followed by a long term leg plaster or external fixation of up to 20 weeks and late weight bearing. He found that a large number of his patients had some form of long term disablement. The Swiss national accident insurance had to pay compensation for partial disablement to approximately one third of patients with tibial fractures, two thirds of those with femur fractures, and to nearly all those with fractures of the weight bearing joints. He decided that the method of fixation was at fault, and considered that early pain free mobilisation might prevent the disablement that was occurring. He started a programme of immediate surgical internal fixation by "plate" or "nail" of fractures to improve the results of fracture care, together with, anatomical reduction, that is the realignment of the broken bones, referred to as osteosynthesis. This therapy was associated with early pain-free active mobilisation, especially during the first 10 days of injury in those patients studied. He noted an immediate improvement in operative complications and a reduction in pulmonary failure. Unfortunately the results of this study made little impact in the Anglo-Saxon world, as the details of such studies were published in his native German (Mueller et al. 1963), and not in English. In Germany, Austria and Switzerland a group was established in 1958 to study early osteosynthesis. As a result of this work, immediate internal fixation of femoral fracture was undertaken in several centres in the late 60's and early 70's. This practice was usually carried out in opposition to the traditional or "conservative" surgical authorities. Although these workers undertook early osteosynthesis to prevent permanent bone injury, they had in fact established a system of preventative action to reduce the incidence of respiratory failure and MSOF. Those workers who continued with the

conservative method of bone fixation unwittingly allowed ARDS and MSOF to develop.

The first such centres to practice early osteosynthesis were in Basel, Helsinki and Seattle. Ruedi and Wolff in 1975, Riska et al. in 1976 and Wolff et al. in 1978 reported that adding immediate internal fixation of femur fractures to the traditional surgery drastically reduced problems of traumatic pulmonary failure and post operative care. Unfortunately none of these early reports used an injury severity score as a method of grading the total body trauma to match the different study groups.

Meek et al. in 1980 in abstract and in a full paper in 1986 and Goris et al. in 1982 started grading the severity of injury with the injury severity score (HTI-ISS). More importantly, they showed that late septic deaths occurred in one of twenty one from the group with early internal fixation of fractures. Fourteen of forty nine patients who had conservative fracture management died of late septicaemia.

In 1985 Johnson et al. and Seibel et al. published similar findings but in better matched groups with more statistical evaluation of the basic data. Both of these studies showed that MSOF and late septic death occurred only in the group with conservative management. They especially showed that deaths from trauma were not related to the magnitude of the original injury as judged by HTI-ISS but to the initial method of management of the injuries.

All of these studies have one critical defect, fracture management was not prospectively randomised with injury severity scores. The study of 178 patients by Bone et al. in 1989 rectified these faults. They were prospectively randomised to either an early stabilisation group, the first twenty four hours, or a late stabilisation group, more than forty eight hours after injury, summarised in Table 24. All urgent surgical procedures were done immediately with only the femoral fracture being randomised according to early or delayed fixation. There were two deaths in the early

treatment group, one from brain injury and the other ARDS. The ARDS patient had an HTI-ISS of 66. There was one death in the late treatment group from ARDS with an HTI-ISS of 26. An ISS of 66 is very severe whereas that of 26 is considered to be only moderate. This work showed unequivocally that early debridement and fixation of the broken bone, especially the femur, considerably reduces the risk of complications.

Table 24. Comparison of early and late stabilisation in traumatised patients. (Bone et al. 1989)				
Treatment group	n	HTI-ISS	ITU days	Average cost \$
Early stabilisation	46	31.8	2.8	19,854
Late stabilisation	37	31.3	7.6	32,915

Historic perspective

Civilian practice in the United Kingdom has adopted a more conservative approach to bone fixation. Three methods of stabilising fractures were employed, on what appears to be a random basis, internal plating, external fixation or traction. Traction and plaster is, and has been, the most popular method of resetting a broken bone. It avoids the necessity of an operative procedure if the bone was plated or pinned. Consequently when the femur is broken, with its high risk of associated MSOF as a complication, it is also often put into traction and plastered.

The work of Smith and Sage (1957) in the USA concluded that the time and method of fixation was unimportant. This work was then cited as evidence supporting conservative therapy. But it should be emphasised that their work was on the forearm, not the femur. So the work's relevance to ARDS and MSOF is inappropriate.

In 1959, Smith published evidence that a delay in fixation actually reduced the incidence of non-union of fractures. Reports by Charnley and Guindy in 1961, Smith in 1964 and Lam in 1964 also showed that late fixation

(greater than 7 days) allowed better union of the fractured bone than early fixation. Standard therapy then became traction of the limb from 7-35 days followed by internal fixation. These reports never addressed the problem of respiratory insufficiency and predate the description of ARDS. It was considered that, at the time of admission, the multiply traumatised patient was too sick for any additional surgery beyond the immediate life and limb saving surgery. Because of the multiple organ systems involved in trauma, most surgeons relegated the care of the fractured limbs to a low priority. Except for debridement of the open wound, definitive fixation of fractures was delayed until the patient's overall condition was stabilised. Debridement of closed fractures was rarely performed.

This early work on fracture stabilisation is highly suspect. It was never randomised and the groups were never matched for severity of injury. Patients receiving early fixation were usually the most severely traumatised, and the period of so called early fixation was between 24 hours and 6 days after the injury. Consequently in the UK there is presumably a greater risk of MSOF in traumatised patients than on continental Europe where early stabilisation and fixation is practised.

Experimental multi-trauma on animals.

Ingvar Jansson, (1982) when working on missile trauma in pigs at the Swedish army research centre, discovered a direct relationship between tissue damage and pulmonary dysfunction.

He divided pigs into 3 groups.

Group 1: 12 pigs were subjected to missile trauma (impact velocity of 450 m/s) in a hind limb. The wound was debrided and the shaft of the tibia and fibula cut with a surgical saw. The fractures were left untreated and not immobilized.

Group 2: 10 pigs were treated in the same way, but the fractures immobilised by internal fixation with a compression plate.

Group 3: 4 pigs were prepared in the same way but no trauma was inflicted.

All the animals were anaesthetised and monitored, and sacrificed after 72 hours. In group 1, 10 pigs developed x-ray changes similar to those seen in patients with ARDS, none of group 2 or 3 showed these changes. Microscopic examination of postmortem tissue showed changes only in group 1. They had interstitial and alveolar oedema, leucostasis and occlusion of capillaries, similar findings to those seen in ARDS. The lungs of group 1 pigs weighed 30% more than those of groups 2 and 3 (16.4 g/kg compared to 10.5 g/kg body weight).

Aseptic conditions were maintained throughout the study and no signs of sepsis were seen. This work shows that ARDS can develop as a result of tissue trauma in the absence of any infection. Tissue damage over a period of hours appears to be necessary to provided a focus upon which the cascade of events can occur, resulting in the ultimate destruction of pulmonary tissue.

They (Jansson et al. 1982) also studied the effect of shooting missiles of differing impact velocities into the legs of 3 anaesthetised pigs. The fracture was left untreated and the study terminated at 72 hours, as shown in Table 25.

Table 25. Effect on lung weight of increasing impact velocity. (Jansson et al. 1982)			
Impact velocity (m/s)	Energy uptake (J)	lung weight (g/kg)	Platelets (10 ⁹ /L)
945	233	10.2	172
1276	439	12.9	96
1500	617	14.4	50
J= joules			

The broken leg was neither debrided or fixed resulting in pulmonary damage similar to that Jannson et al. (1982) previously described. The degree of pulmonary damage was proportional to the amount of energy imparted to the tissue. The weight of the lungs increased, due to pulmonary oedema, with the increasing impact velocity. Blood platelet count also fell in a similar manner.

The work of Jannson showed that although multi-system organ failure was commonly attributed to bacterial infection, inflammation and tissue damage may be the initiators of this condition.

Thorne et al. (1989) studied neutrophil sequestration in an anaesthetised pig trauma model. Autologous neutrophils from 10 pigs were labelled with radio-labelled Indium-oxine. They were subjected to trauma by striking both hind limbs 100 times with a 10 pound mallet. Although there was a significant fall in blood pressure it was not profoundly hypotensive and quickly returned to normal levels. This fall in blood pressure was insufficient to cause bacterial egress from the intestinal lumen. Within 1-3 minutes of trauma, the radioactivity over the lungs increased dramatically, indicating pulmonary neutrophil sequestration. This was followed by a small decrease after 90 minutes, but the levels remained significantly raised. Liver neutrophils sequestration also showed a significant increase, but there was marked reduction in neutrophil activity in the heart. This work shows unequivocally that neutrophil aggregation occurs in the lung and liver in the absence of any infection or endotoxic stimulus. Damaged tissue in the hind limb provides sufficient inflammatory response to initiate a whole body response.

Conclusion.

Trauma is an important cause of ARDS and MSOF, especially when associated with fracture of the femur. The high energy level required to break the femur causes considerable damage to the muscle. The tissue

mass of the contused quadriceps provides an inflammatory focus large enough to initiate whole body inflammation. If the femur is not set and debridement performed within 24 hours, then secondary tissue damage resulting from the inflammatory response can occur. The septic syndrome can then develop without the involvement of infection, although infection may develop as a complication. In these circumstances ARDS and MSOF are almost inevitable.

Unfortunately the early fixation of broken bones and debridement of damaged tissue is not a common practice. More popular is to wait and see. Another popular treatment of multi-traumatised patients is to wait for them to improve before fixing their broken bodies.

All multi-trauma produces respiratory distress although it might often be sub clinical. Fat embolism is not a very common cause of respiratory distress.

Section 3.

Oxygen Deprivation.

Introduction.

The concept of oxygen deprivation as a cause of ARDS and MSOF is a relatively new one. Major injury such as sepsis, trauma, pancreatitis, burns or major surgery, all cause a significant increase in the metabolic rate. Those patients who are unable to raise their metabolic rate could be at risk of developing MSOF. This increased rate must have a higher oxygen consumption than the basal level would allow. The purpose of this increased VO_2 is to allow an increased production of ATP. Unfortunately these major injuries are often associated with a reduced VO_2 , partly due to the decrease in oxygen delivery to the tissues. Additionally these injuries can cause a redirection of blood flow away from certain organs causing tissue ischemia. Although oxygen delivery may appear normal or even raised it is in fact lower than that necessary to maintain the integrity of certain tissues.

The functional tissue ischemia can prevent the adequate formation of ATP and allow the production of hypoxanthine. Hypoxanthine via the enzyme xanthine oxidase can allow the production of super oxides and hydroxyl ions. These compound are widely known to cause tissue damage. Such tissue damage would cause the inflammatory reaction to commence, and if substantial enough could lead to the development of whole body inflammation.

Oxygen metabolism

Introduction

Oxygen transport by arterial blood is defined in terms of the total oxygen delivered to the tissues, oxygen delivered (DO_2). The total amount of oxygen consumed by the tissues is the oxygen consumption (VO_2) and the ratio between the two, the oxygen extraction ratio (O_2ER). Oxygen delivered

is the product of the volume of oxygen carried by arterial blood, the arterial oxygen content (CaO_2) and the cardiac output (CO):

$$\text{DO}_2 = (\text{CO}) \times (\text{CaO}_2)$$

To compare different patients, this is usually indexed to the body surface area (BSA) to give the oxygen delivery index (DO_2I):

$$\text{DO}_2\text{I} = \frac{\text{CO} \times \text{CaO}_2}{\text{BSA}}$$

The units are $\text{ml}/\text{min}/\text{m}^2$.

The oxygen consumption index (VO_2I) is the difference between the arterial and the venous oxygen content (CvO_2) multiplied by the cardiac output:

$$\text{VO}_2\text{I} = \frac{\text{CO} \times (\text{CaO}_2 - \text{CvO}_2)}{\text{BSA}}$$

The arterial oxygen content is usually derived from the arterial oxygen saturation (SaO_2) the haemoglobin (Hb) concentration and 1.34, the number of ml of oxygen carried per gram of haemoglobin per 100 ml of blood:

$$\frac{\text{CaO}_2}{100\text{ml}} = \text{SaO}_2 \times \text{Hb} \times 1.34$$

So that:

$$\text{VO}_2\text{I} = \frac{\text{CO} \times (\text{SaO}_2 - \text{SvO}_2) \times \text{Hb} \times 13.4}{\text{BSA}}$$

The oxygen extraction ratio is the ratio of the delivery to the consumption:

$$\text{O}_2\text{ER} = \frac{\text{VO}_2\text{I}}{\text{DO}_2\text{I}}$$

and is usually expressed as a percentage.

The basic determinant of human life is the ability of cellular metabolism to maintain oxidative phosphorylation. The body has to distribute oxygen according to need because a prerequisite to its utilisation is the transport of oxygen to the cell.

$$\begin{array}{ccccc} \text{Uptake} & = & \text{Delivery} & \times & \text{Extraction} \\ (\text{organ}) & & (\text{resistance vessels}) & & (\text{exchange vessels}) \end{array}$$

If the whole body is used to assess oxygen consumption (VO_2), then a form of the Fick equation is easy to apply (reverse Fick principle):

$$\begin{array}{ccccc} \text{VO}_2 & = & (\text{CO} \times \text{CaO}_2) & ((\text{CaO}_2 - \text{CvO}_2) / \text{CaO}_2) \\ (\text{oxygen consumption}) & & (\text{delivery}) & & (\text{extraction}) \end{array}$$

Oxygen extraction ratio in normal patients

Under normal circumstances, a reduction in DO_2 or an increase in VO_2 is accompanied by an increase in the amount of oxygen extracted from the blood, resulting in a widened arterio-venous oxygen difference. Patients with a variety of acute illnesses are unable to increase their O_2ER despite marked reductions in DO_2 . The cause of this is unclear.

A poor O_2ER has been ascribed to several circulatory defects, the peripheral shunting of blood flow through arterio-venous connections in an organ, the inability to recruit enough capillaries to utilise delivered oxygen, the inappropriate distribution of blood flow to organs, poor oxygen diffusion to the cell from the capillaries or damage to the mitochondria.

The relationship between DO_2 and VO_2 .

In healthy subjects, normally those performing vigorous exercise, VO_2 is maintained despite a drop in oxygen delivery (DO_2) as the O_2ER increases proportionately. However, below a critical DO_2 , any further decrease in DO_2 produces a proportionate decrease in VO_2 , and lactic acid accumulates. This critical DO_2 threshold has been estimated as about 8ml/kg/min . At this point the O_2ER is about 70%.

Historically, ie. before ITU measurements of DO_2 and VO_2 became commonplace, it was assumed that VO_2 was independent of DO_2 . It was thought that the metabolic rate was the primary determinant of VO_2 , not the rate of oxygen supply. It is now appreciated that VO_2 is dependent on DO_2 to the organs of the body, the distribution of CO to those organs, the density of capillaries within those organs and the metabolic needs of those organs. The oxygen delivery to any organ will depend on its share of the cardiac output. The extraction of oxygen from that delivered is determined by the needs of cellular metabolism and the ability of the organ to change the number of capillaries being perfused. Vaso-regulation controls distribution,

and resistance vessels must dilate to increase flow, possibly in response to oxygen need.

When oxygen requirements are high, increased demand can be met by increased delivery, through increased CO, and increased extraction through increased capillary density. Increases in cardiac output can be accommodated by altered resistance. When the oxygen content of arterial blood is low, either flow must be increased or extraction must be increased yet further. In pathological states there may not be an appropriate match between oxygen supply and oxygen demand, that is those organs with the greatest need may not receive an adequate supply. Those organs with lower needs may have an inappropriately high flow. There may also be an inability of the body to extract the required oxygen.

The arterial/venous oxygen difference is dependent on the relative distribution of blood to the various organs and the effective capillary density of the organ. Skin, gut, kidney and muscle are considered flow independent organs; they have high blood flows that are not primarily for the delivery of oxygen. Flow dependent organs are the brain and heart in which the blood flow is primarily for the delivery of oxygen. Blood flow in the skin, gut or kidney is not as important in ATP production and VO_2 as flow to the heart or brain. If there is a high blood flow to the skin or muscle in a moribund patient, then less oxygen will be consumed than if there is a high flow to the heart or brain. If the effective capillary density of an organ is low then the O_2ER of that organ will be reduced. The capillary density of an organ changes according to its need, by activating pre-capillary sphincters that are sensitive to hypoxemia and its metabolites eg. exercising muscle has a higher effective capillary density than resting muscle. When the capillary density of an organ is increased the oxygen extraction ratio can be raised, and a greater proportion of the oxygen delivery to the organ will be consumed.

Relationship of DO_2 to VO_2 in normal patients.

In healthy persons VO_2 is independent of DO_2 . Any reduction in DO_2 results in compensatory mechanisms to satisfy tissue demand. These adaptations include increased O_2ER , increase in local capillary perfusion and shifts in the oxyhaemoglobin dissociation curve.

Shibutani et al. (1983) studied 58 patients undergoing coronary artery by-pass graft operations. The relationship between DO_2 and VO_2 was studied in each patient.

They produced data sets for each patient and found that there was a critical value for DO_2 of 330 ml/min/m^2 when VO_2 was 109 ml/min/m^2 . Below this value VO_2 decreased proportionately showing a linear relationship between DO_2 and VO_2 , demonstrating that VO_2 was supply dependent. At higher values of DO_2 , VO_2 showed no further increase, and a plateau was reached when it became supply independent.

In 15 patients DO_2 levels less than 330 ml/min/m^2 were observed and the plasma lactate concentration showed lactic acidosis. Whereas those patients having a DO_2 higher than 330 ml/min/m^2 had normal lactate levels. The decrease in VO_2 seen during surgery was previously attributed to decreased metabolic demands, caused by anaesthetic agents (Ngai and Papper 1962), but now a reduction in DO_2 below 330 ml/min/m^2 is interpreted as evidence of tissue oxygen deprivation. Waxman et al (1982) reported an association of decreased intraoperative VO_2 with increased post-operative lactic acidosis, showing that the reduced VO_2 during anaesthesia and surgery reflects inadequate tissue oxygenation. Patients who are supply dependent, exhibit an oxygen deficit, as can be seen in Table 26.

This important piece of work by Shibutani et al. (1983) was considered to show the relationship between DO_2 and VO_2 . It reflected the normal relationship between DO_2 and VO_2 and could be extrapolated to normal

subjects. Oxygen consumption is independent of DO_2 until a critical low point is reached when VO_2 becomes flow dependent.

Table 26. Haemodynamic parameters associated with a DO_2 less than and greater than 330 ml/min/m^2 in 58 patients. (Shibutani et al. 1983)		
Parameter	$\text{DO}_2 < 330$	$\text{DO}_2 > 330$
MABP	85	85
CI	1.7	2.5
DO_2	273	450
VO_2	86	109
O_2ER	31	24
Lactate mMol/l	14.9	10.9
PvO_2	38	42
SvO_2	70	75

MABP mean arterial blood pressure, CI cardiac index, PvO_2 oxygen partial pressure in venous blood, SvO_2 oxygen saturation of venous blood

The basal VO_2 during surgery was previously reported by Guedel (1924) to be 138 ml/min/m^2 . The work of Shibutani et al. (1983) showed a plateau value of 109 ml/min/m^2 and is therefore 78% of basal level. The 22% reduction in the mean value of VO_2 was thought by Shibutani et al. (1983) to reflect the reduced oxygen requirement produced by anaesthesia and the lower temperature seen in these patients during surgery.

The data shown in Table 26 show that when DO_2 was below the critical value of 330 ml/min/m^2 , then there was a compensation in the O_2ER , which rose from 24% to 31% to maintain its VO_2 . The reduced oxygen partial pressure and saturation in the venous blood shows that there has been a greater extraction of oxygen.

Pepe and Culver (1985) performed studies on 21 dogs to measure the relationship between DO_2 and VO_2 . They divided the dogs into three groups

to reduce the DO_2 : group 1 had DO_2 reduced by increased positive end expiratory pressure (PEEP), group 2 had flow reduced by venous obstruction and group 3 had DO_2 reduced by increasing PEEP and initiating lung damage. In all three groups, VO_2 was independent of DO_2 above a critical value of 13ml/kg/min . (workers using animals adopt body weight as an index of flow instead of body surface area). Below this critical value VO_2 was linearly dependent on DO_2 .

These two important studies show that in the whole body, VO_2 is dependent on DO_2 when below the critical value, but is independent, forming a plateau when DO_2 is above this value. In studies of individual tissue beds this relationship does not occur. This was shown by the work of Stainsby and Otis (1963) on skeletal muscle in dogs.

Stainsby and Otis (1963) looked at the effect of changes in blood flow on oxygen consumption, of the *in situ* gastrocnemius-plantaris muscle group, in dogs. When perfusion pressure was reduced, the flow decreased. The reduction in blood flow was matched by an equivalent increase in the arterio-venous oxygen difference. As a result the calculated VO_2 was unchanged. Some remarkably low levels of venous oxygen content were seen during the period of reduced flow. Even though the venous oxygen saturation fell as low as 10%, representing a venous PO_2 of about 10 mmHg, there was no decrease in VO_2 . When blood flow was increased to high levels there was still no change in VO_2 . Stainsby and Otis (1963) concluded that VO_2 was independent of DO_2 except at very low, minimal or critical levels. They thought that the rate of oxygen uptake of resting skeletal muscle in these experiments, was regulated by some factor other than the amount of oxygen delivered by the blood. Stainsby and Otis (1963) also subjected the skeletal muscle to contraction and showed an increase in the O_2ER . They applied the Krogh equation to estimate capillary density in these studies. The resting muscle was calculated to have a capillary density of $40/\text{mm}^2$. The resting muscle with reduced blood flow was estimated to have an

increased capillary density of $150/\text{mm}^2$. Contracting skeletal muscle during progressive hypoxemia had a considerably increased density of about 700 capillaries/ mm^2 . Capillary density for this group of muscles has previously been estimated histologically (Schmidt-Nielsen and Pennycuik 1961) and found to be about $750/\text{mm}^2$.

Inadequate capillary recruitment

Extensive studies were performed by Granger et al. (1975) on the control mechanisms of capillary blood flow and oxygen extraction. In 36 dogs they looked at the effects on hind limb vasculature, in the whole animal, of the following interventions:

- a) Arterial hypotension of 80 mmHg.
- b) Thigh muscle contraction by electrical stimulation.
- c) Arterial hypoxia by respiring 6% oxygen.
- d) Adrenergic stimulation by noradrenaline to increase vascular tone and reduce blood flow and venous oxygen levels.

In Table 27, under the column headed "control B", are data recorded before hypotension was induced, whereas "control A" shows data recorded when reducing the blood pressure by 20 mmHg. There was a fall in blood flow of 50%, but in spite of the reduction, normal VO_2 was maintained, suggesting that the O_2ER increased twofold. Associated with this increase in O_2ER was a doubling of the capillary filtration coefficient, suggesting a doubling in the number of perfused capillaries. With hypoxemia, in column B, before hypotension was induced, the blood flow was higher than corresponding control column B. This suggests that autoregulation was occurring to maintain VO_2 . But when hypotension was introduced, as seen in column A, there was a small fall in blood flow, accompanied by an increase in O_2ER as shown by the rise in capillary density.

With muscle contraction, before hypotension, in column B, there was an increase in blood flow accompanied by a rise in capillary density and A-V

oxygen difference, compared to control value, in column B. The large increase in VO_2 from 4.4 to 18 reflects the increase in ATP production. Hypotension causes a further increase in capillary density to 0.033, this is a 150% increase in capillary density as reflected by Krogh's coefficient.

Table 27. Action of hypotension alone and in combination with either hypoxemia or muscle contraction on muscle of dogs. (Granger et al. 1975)						
	Control		Hypoxemia		Contraction	
	B	A	B	A	B	A
BP mmHg	100	80	100	80	100	80
A-V O_2 (vol%)	4	9	3	4	10	12
Flow ml/min	100	50	130	115	140	135
VO_2	4.4	4.0	4.2	4.2	18	17.6
Pv O_2	45	30	32	28	24	22
Kf	.013	.020	.020	.032	.026	.033

B = before hypotension, A = after hypotension, Kf = Kroghs' capillary filtration coefficient (capillary density)

In Table 28, hypoxemia alone, was found to increase blood flow and raise the capillary density, suggesting an increase in O_2ER , so that VO_2 could be maintained. But when noradrenaline was added to the preparation there was a precipitous fall in blood flow that was compensated for by a large increase in the capillary density. When hypoxemia was initiated in these conditions there was a large increase in blood flow and capillary density.

Table 28. The effect of hypoxemia, on noradrenaline treated muscle, in dogs.
(Granger et al. 1975)

	Control		Noradrenaline	
	B	A	B	A
BP mmHg	100	100	100	100
A-V O ₂ (vol%)	6	2	13	2
Flow ml/min	80	200	35	160
VO ₂	3.5	3.7	3.7	3.3
PvO ₂	42	21	24	20
Kf	.008	.018	.027	.036

B = before hypoxemia, A = after hypoxemia

Although blood flow decreased during hypotension and contraction via electrical stimulation, effective capillary density consistently increased, suggesting an unexpected relaxation of the vascular structures involved in the regulation of exchange activity. These findings suggest that the resistance vessels escape from the adrenergic constrictor action, when tissue oxygenation was lower than normal. Although muscle cell PO₂ was maintained within narrow limits despite a variety of local stresses, nervous control of vascular resistance in skeletal muscle is of major importance for the reflex regulation of systemic arterial pressure.

When muscular blood flow was reduced by the vasoconstrictor, noradrenaline, oxygen delivery to the exchange vessels was reduced. The low level of oxygen delivery to the tissues stimulated the metabolically sensitive sphincters to dilate, overriding the adrenergic constrictor effect. The effective capillary density rose and maintained the VO₂. This increase in diffusion capacity compensated for the reduction in blood flow and increased the O₂ER. Thus there was an interaction of local and nervous control to regulate arterial pressure and DO₂. Oxygen metabolism appears to exert the greatest influence on capillary density.

Cain (1978) looked at the whole body effect of oxygen metabolism. He demonstrated a reduced O_2ER in hypoxic dogs when treated with the alpha-blocking drug phenoxybenzamine. He studied the effects of vasoconstrictor tone on oxygen extraction in 2 groups of anaesthetised dogs, by ventilating them on 9% oxygen. Group A were given phenoxybenzamine to produce alpha blockade, group B were left untreated as controls. He measured DO_2 , VO_2 and pulmonary artery oxygen saturation. Alpha blockade prevents the venoconstriction that would normally occur in hypoxemia. Group A with a low systemic vascular resistance were never able to take up as much oxygen as group B. The venous blood from group A always contained more oxygen than group B. In group A, the oxygen delivery and uptake systems became less efficient as a result of the loss of vasoconstrictor tone. He was able to show that group B were better able to use the low volume of oxygen delivered to the tissues. The VO_2/DO_2 curve was much steeper in group B than group A. Group A had a lower VO_2 than group B for the same DO_2 . It was thought that local vasoconstriction allowed the diverting of blood flow from tissue of low oxygen demand to those of high demand. Cain was also able to show that in group B, VO_2 considerably improved after 10 minutes of hypoxemia, this characteristic was never seen in group A.

He considered (Cain 1978) that flow independent organs like the kidney, skin and gut would normally vasoconstrict to redistribute blood to those flow dependant organs such as the brain and heart. This, of course, could not occur in alpha receptor blocked animals. Higher venous blood saturation and low SVR seen in group A is also seen in ARDS and MSOF.

Cain's work shows that differing organs have individual responses to oxygen shortage. When they are forced to behave in a similar manner by drug therapy, such as alpha receptor blockade, the whole body becomes less efficient at extracting oxygen.

Table 29. Distribution of blood flow and oxygen utilisation in various organs, of control dogs. (Taylor et al. 1987)

	Q	CO	Q/kg	VO₂	Q/VO₂	A-V
Brain	840	14	600	52	16	6.2
Heart	300	5	1000	34	9	11.4
Splanchnic	1680	28	646	83	20	4.9
Kidney	1380	23	4600	19	72	1.4
Skeletal muscle	960	16	31	57	17	6.0
Skin	480	8	133	12	40	2.5

All parameters are expressed as ml/min, except CO which is the percentage of CO going to the particular organ.

Q = blood flow

VO₂ = oxygen consumption

Q/VO₂ = oxygen utilisation ratio

A-V O₂ = arterio-venous oxygen content difference

Table 29, published by Taylor et al (1987), shows the different blood flows and oxygen utilisation patterns in various organs. Note the large difference between the oxygen utilisation ratio in different tissues relative to flow (Q/VO₂). The heart has a ratio of only 9 whereas the kidney's ratio is 72. The heart utilises most of its available oxygen, while the kidney has an abundance of blood flow relative to utilisation. The heart will need an increase in blood flow if its oxygen needs increase (supply dependant), while the kidney can simply extract more oxygen from its abundant blood supply, without requiring a change in flow (supply independent). Measurement of differing organ needs demonstrates that the total body oxygen consumption is a poor indicator of actual requirements in individual tissue beds.

The inappropriate distribution of cardiac output with excessive flow to tissues of low metabolic demands (eg. kidney gut and skin), reducing the

flow to those organs with high demands (brain or heart) would result in a reduced O_2ER . A fall in VO_2 for any given DO_2 would occur. This fall in VO_2 is also seen in septicaemia and MSOF.

Effective capillary perfusion

The local microvascular tone or systemic vascular resistance is controlled at two levels. The first are the small muscular arterioles, the major systemic resistance vessels, controlling overall blood flow into the tissue bed. They appear to be most active at low tissue PO_2 , dilating to permit increased DO_2 .

The second, but major focus of control, at more modest levels of hypoxemia, are the precapillary sphincters which regulate the number of open capillaries and thus the capillary density, optimising the conditions for gas diffusion, allowing changes in the O_2ER .

Regional shunting of organ blood flow.

When a low O_2ER or low oxygen uptake is seen clinically, there is an assumption that blood is by-passing the capillary bed of an organ via an anastomosis of the arterioles and venules, preventing the perfusion of nutrient capillaries. This arterio-venous blood flow, by-passing the organ's capillary bed, is referred to as shunting. But the evidence for the existence of an A-V shunt is very limited. Archie (1976) looked at shunting by injecting radio labelled microspheres into three groups of dogs: controls, animals infused with endotoxin, and animals made septic or developed septic shock. Mean shunting was 7.7% in control dogs, 7.3% and 4.3% respectively in the endotoxic and septic animals. Regional shunting in the brain, heart and skeletal muscle were not significantly different. However mean shunting in the splanchnic circulation was 36.5% in the septic animals and 18.6% in the control group, a significant difference. Renal shunting in the endotoxic group was 15%, but 4% in the controls and septic group. Regional shunting

is therefore considered to be of little consequence, except in the splanchnic region during sepsis.

Increased Oxygen Consumption

Patients at risk of developing ARDS or MSOF from infection, multi-trauma, burns, major surgery and other insults would be expected to have an increased metabolic rate and oxygen consumption. Much of the increased oxygen consumption is in the splanchnic circulation. It is likely as Shoemaker suggests that unless this increased need is met, perfusion of other organs will suffer and hypoxic damage may result.

Cuthbertson as long ago as 1932 measured the VO_2 of patients suffering leg trauma and found that oxygen consumption was 20-25% higher than that found in patients in the basal condition.

Critically ill patients often have an increased temperature even in the absence of infection. Hyperthermia would be expected to increase the metabolic rate and oxygen consumption. Burns patients have been studied extensively and have a considerable increase in metabolic rate. The degree of increase is proportional to the percentage of the skin surface burned. The increase in basal metabolic rate ranges from 25% for a 10% burn to 115% for a 75% burn (Wilmore et al. 1974). Patients with fractures have an increase in basal metabolic rate of 20-25% (Cuthbertson 1932), peritonitis patients 5-25%, and severe infection and multiple trauma 30-50% (Duke et al. 1970).

Regional oxygen consumption in 15 patients with intraperitoneal infections was investigated by Gump et al. (1970) They looked at cardiac output, splanchnic bed blood flow, VO_2 and splanchnic bed oxygen consumption. Resting VO_2 was within normal limits in 6 patients, but was raised by 19-44% in 9 others. In those 9 patients, oxygen consumption by the splanchnic bed was 26% of total VO_2 , and 51% of the increased VO_2 . They showed that 42% of the extra CO was accounted for by the splanchnic bed. Increased VO_2 was associated with increased CO in all patients, there was

no tendency to increase O_2ER to meet the additional oxygen needs, as can often be seen in exercising athletes.

Similar findings were reported by Wilmore et al. (1980) when they looked at burns patients. Hepatic blood flow increased by 150% and hepatic VO_2 increased by 30%, while lactate uptake increased by nearly 200%. These patients did not show lactic acidosis. Splanchnic VO_2 normally 22% of VO_2 rose to 30%. These data show that the hepatic metabolic rate had increased to maintain homeostasis by increasing blood flow and oxygen consumption.

Dahn et al. in 1987 looked at splanchnic and total body VO_2 in 12 septic and 7 nonseptic but injured patients, shown in Table 30. Patients with sepsis had a splanchnic VO_2 that was 42.9% greater than patients without sepsis. Splanchnic VO_2 was 30.2% of total VO_2 in nonseptic patients and 43.8% in septic patients. Cardiac index was 27% higher in septic patients compared to nonseptic. The septic group had a splanchnic blood flow index 22% greater than nonseptic patients. The proportion of cardiac index flowing to the splanchnic bed was similar in both groups.

The proportion of splanchnic VO_2 to total VO_2 for septic patients is disproportionately large compared to nonseptic patients. Forty four percent of total VO_2 is consumed by the splanchnic bed compared to 30% in the nonseptic patients. Although the cardiac index and DO_2 is significantly higher in the septic group, VO_2 is lower, showing an impaired extraction ratio. But the extraction fraction in the splanchnic bed was not significantly different when comparing the two groups.

Table 30. Oxygen utilisation dynamics in septic and nonseptic patients.

(Dahn et al. 1987)

	Nonsepsis (n=7)	sepsis (n=12)
Total body oxygen consumption ml/kg/min.	4.39	4.34
Splanchnic oxygen consumption ml/kg/min.	1.33	1.90*
Cardiac index L/min/m ²	3.91	4.98*
Splanchnic flow index L/min/m ²	1.11	1.35
Systemic extraction fraction %	32	23*
Splanchnic extraction fraction %	32	39
Mixed venous oxygen tension	34	38.7
Hepatic venous oxygen tension	33.7	29.9#
Haematocrit %	34.9	33.9
Arterial lactate (mEq/L)	0.87	0.59
Splanchnic lactate uptake (μEq/kg/min)	10	18

* difference significant at $p < 0.05$ compared to nonseptic,# difference significant at $p < 0.01$ compared to mixed venous.**Supply dependency of oxygen consumption in ARDS patients.**

In humans and animals previously described, the level of oxygen uptake was shown to be independent of oxygen delivery, down to a critical level of delivery, below which there exists a direct relationship between these two variables. This relationship was further evaluated by looking at patients sufferings from ARDS.

Danek et al. (1980) looked at the relationship of DO_2 to VO_2 in 20 ARDS and 12 non-ARDS patients. In 11 ARDS patients, group 1a, they manipulated DO_2 , by altering the pressure of inspiration on the PEEP ventilator, until a maximum level was achieved, and DO_2 reduced. The data was collected over 2-6 hours with 30 minute stabilisation periods. In the

other 9 ARDS patients, group 1b, data was collected during the acute phase of their illness, which was a longer period than group 1a, 3-9 days. But it became apparent to Danek et al. (1980) that DO_2 and VO_2 patterns were similar in the two groups. The 12 non-ARDS patients were critically ill and had DO_2 's that would be expected to vary. Eight of these patients were mechanically ventilated, but none required PEEP. Measurements were made twice daily for 2.5 -7 days.

In the ARDS patients there was an increase in VO_2 with DO_2 over the whole range of values, suggesting a direct relationship between DO_2 and VO_2 . The DO_2 did not reach a critical point or plateau and the O_2ER when calculated from these data was a constant 22%. Danek et al. (1980) suggested there were two important conclusions from these finding; The first that even at low DO_2 , the O_2ER does not increase to maintain VO_2 and secondly, that even at high levels of DO_2 , VO_2 continues to rise i.e. VO_2 is flow dependent at all levels.

Observation of the data presented by Danek et al. (1980) on the relationship between DO_2 and VO_2 shows most of the data points to be quite high. Only 5 points are below a DO_2 of 8 ml/kg/min. The other 28 points are between 8-30 ml/kg/min. Consequently it is impossible to observe if there is an increase in O_2ER at a low level of DO_2 . Cardiac output in ARDS patients was 8.2 l/min, whereas in non-ARDS patients it was 5.6 l/min. Although the CO in both groups are relatively high, the venous blood oxygen saturation in ARDS patients of 39% and 17% in non-ARDS, are low in ARDS patients and extremely low in non-ARDS patients. Normal subjects should show about 70% saturation. The conclusion from this set of data must be that although the ARDS patients are receiving a high DO_2 , it is not yet high enough. The ARDS patients show supply dependency because of their very high demand. The non-ARDS patients showed no relationship between DO_2 and VO_2 .

Mohsenifar et al. (1987) studied 10 patients with ARDS to determine the relationship between VO_2 and DO_2 , by modifying the pressure of

inspiration during PEEP ventilation. Mohsenifar et al. (1987) did not make any measurements on a control population of patients, so we do not know what variations are due to their techniques of data collection.

Below a critical DO_2 of 21 ml/kg/min, VO_2 was linearly related. The CO averaged at 7.1 l/min with a venous blood oxygen saturation of 32%. This shows that although the CO was reasonably high, the saturation was low, suggesting that the CO was not high enough. This again suggests that ARDS patients have a far greater oxygen need than is normally seen. However at a higher DO_2 they were unable to show any correlation with VO_2 or the development of a plateau. It should be mentioned that Mohsenifar et al. (1987) only showed 8 data points higher than a DO_2 of 21 ml/kg/min. There were about 90 data points, mostly below 21 ml/kg/min. Conclusions based on 8 data points from 10 patients seems to be stretching credibility. The O_2ER calculated from the numerical data, below their critical point, was 27%, but from the graphical data was 33%. It should be mentioned that the O_2ER seen in ARDS patients is similar to that seen in normal subjects.

Annat et al. (1986) failed to observe a supply dependency of VO_2 in ARDS patients, the reason for this was most likely the high DO_2 seen in most of his patients. This was reflected in the normal blood lactates observed in his patients. Similar flow independency was also found by Pepe and Culver (1985) in experimental ARDS in dogs, also due to the high level of DO_2 .

Oxygen debt in septic patients.

Kaufman et al. (1984) treated 8 patients with septic shock and 5 with hypovolaemic shock. They used fluid resuscitation to increase CO and DO_2 , giving a fluid challenge of 250 ml every 15 minutes until the pulmonary capillary wedge pressure (PCWP) reached 15 mmHg, usually lasting about

70 minutes. Measurements were obtained every 30 minutes during the fluid challenge.

Kaufman et al. (1984) showed in Table 31 the changes in DO_2 and VO_2 in these groups of patients.

Table 31. Changes in oxygen delivery and consumption in patients with septic or hypovolaemic shock, challenged with volume infusions. (Kaufman et al. 1984)				
	septic shock		hypovolaemic shock	
	PE	AE	PE	AE
DO_2 ml/min/m ²	315	424	239	386
VO_2 ml/min/m ²	134	151	96	135
CI l/min/m ²	2.34	3.48	1.56	2.54
PvO ₂ mmHg	42	44	31	35
SVR dyne/s/cm	1207	835	1368	1167
O ₂ ER %	45	36	42	37
Lactate mg/dl	78	68	64	52
PE pre-expansion, AE after expansion				

The increases in DO_2 and VO_2 seen in both groups were significant. There was no significant difference between the increase in DO_2 or VO_2 seen in the septic group or the hypovolaemic group. Increasing DO_2 by fluid resuscitation increases VO_2 in both septic and hypovolaemic shock. Even though there was a significant rise in the CO, the partial pressure of oxygen from venous blood of both groups, although improved, remained low, suggesting that the patients were still extracting a higher than normal volume of oxygen. The lactate levels were also raised in both groups, and although they fell, they were still higher than normal. These data suggest that the raised CO was still too low to meet the oxygen requirements.

This therapy revealed an oxygen debt in both groups of patients. These results also show flow dependency of VO_2 on DO_2 , in both groups of patients. Unlike the findings of Shibutani et al. (1983) flow dependency continues to be seen throughout all the values of DO_2 obtained. The O_2ER , even after therapy was still high, in the septic group at baseline it was 45% falling to 36% and 42% falling to 37% in the hypovolaemic group. If fluid therapy had been maintained to a higher PCWP, then greater DO_2 and VO_2 may have been seen with a further fall in O_2ER . Equally, if the therapy had been maintained for a longer period then benefit to the patients may have been seen. Clearly the therapy caused vasodilation as can be seen by the fall in SVR, but there was a fall in capillary recruitment, reflected by the fall in O_2ER .

The DO_2 and VO_2 obtained after fluid therapy were still lower than those recommended by Shoemaker et al. (1973) and 12 of the 13 patients still died. These patients did not have their DO_2 and CO maintained by this fluid therapy, it was curtailed after the peaks had been achieved. This study shows that there is little pathological difference between the two groups of patients. They were both suffering from hypovolaemia, low CO and DO_2 . These data suggest that there is unlikely to be any cellular malfunction in the septic group, different to the hypovolaemic group, such as mitochondrial impairment.

The work of Mohr et al. (1969) was especially interesting as they studied VO_2 in different groups of septic shock patients when given fluid therapy. They showed a group of patients who did not increase their VO_2 when given fluid. Close examination of their data shows that these patients started with VO_2 of 200 ml/min/m². Whereas the patients who increased their VO_2 with fluid challenge had initial VO_2 of 154 increasing to 217 ml/min/m². This suggests that at lower DO_2 , VO_2 has a direct relationship. But at the higher levels a plateau is reached when VO_2 is independent of DO_2 .

Bihari et al. (1987) suggested that inadequate oxygenation of peripheral tissue may be unrecognised in critically ill patients and may worsen their prognosis. Although they observed the patients before their study they did not optimise their treatment. They measured the oxygen delivery, tissue oxygen uptake and oxygen extraction ratio during a 30 minute infusion of the vasodilator prostacyclin, in 27 critically ill patients with acute respiratory failure. Prostacyclin increased significantly the indexed DO_2 , from 375 to 492 ml/min/m² in the 14 patients who survived and in the 13 who died. This increase in oxygen delivery was accompanied by a significant 19% increase in VO_2 by the patients who died, but only 5% in the survivors. The survivors O_2ER fell by 17%, but in the patients who died, it rose by 11%. These data suggest the presence of a substantial oxygen debt in those patients who subsequently died. It also suggests an increase in capillary recruitment allowing the tissue to increase its O_2ER . There was an increase in O_2ER uncovered in those patients who had been inadequately treated. This work shows the importance of the relationship between cardiac index (CI), DO_2 , VO_2 and O_2ER in ARDS and MSOF.

The work of Bihari et al. (1987) further established the importance of tissue oxygen in the development of MSOF. They showed that the level of VO_2 can differentiate the survivors from the non-survivors.

Defective oxygen extraction ratio in ARDS.

Duff et al. in 1969 studied 22 patients with septic shock. The cardiac index was frequently normal or raised despite symptoms of shock. Systemic vascular resistance and VO_2 were low. When DO_2 increased, venous oxygen content rose, showing an inability of the patient to increase his oxygen consumption.

In septic shock in man, a paradox exists with patients having normal or raised CI, raised lactate and narrowed arterio-venous oxygen content. Raising the DO_2 merely increases the venous saturation, showing that

something has happened to the tissue's ability to utilise available oxygen, i.e., an extraction defect exists as compared to a supply defect. In normal patients, sufficient oxygen can be extracted until delivery decreases to below approximately 8-10 ml/kg/min, but in ARDS patients an oxygen delivery of below 21 ml/kg/min is associated with a decreasing oxygen utilisation.

Optimum values for oxygen metabolism.

Investigation of oxygen metabolism as a mechanism of the pathology of ARDS and MSOF was carried out by Shoemaker et al. (1973). They studied the sequential physiological variables in surgical patients who were in danger of developing complications. They measured many data sets from patients every 30 minutes during surgery and up to 96 hours postsurgery.

Circulatory problems were studied in surgical patients, as this group of patients could have measurements of their numerous parameters made before their surgery occurred. Measurements could also be made during surgery. Unlike other patients who develop ARDS and MSOF, coming into an ITU, surgical patients could have measurements made at known time intervals from the time of insult. Haemodynamic and oxygen transport patterns were observed to characterise the effects of surgical trauma. The patients underwent operations for massive gastro-intestinal bleeding, bowel obstruction and rupture, severe multiple trauma as well as other conditions, but all were high risk, critically ill patients.

They studied 98 patients, 67 of whom survived while 31 died. Arterial blood pressure was found not to be a good indicator of survival, but those parameters associated with volume and oxygen transport, ie. cardiac index, DO_2 and VO_2 , were better indicators. In 1985, Bland in Shoemaker's group, performed a similar study in 220 critically ill surgical patients. They found that the DO_2 in survivors was significantly greater than normal, and much greater than the values found in non-survivors. The non-survivors were unable to achieve these values, while survivors achieved these DO_2 values by

the second post-operative hour. Unfortunately, these higher DO_2 values required greater work by the heart, those patients unable to achieve this higher myocardial performance had a poor likelihood of survival. The non-survivors had a lower cardiac index than survivors at similar left atrial pressures, showing reduced myocardial performance.

Shoemaker et al. (1973) considered that the cause of death was circulatory deficiency, best described as inadequate DO_2 leading to an inadequate VO_2 , with failure to maintain cellular metabolism. Those patients with impaired myocardial performance were unlikely to maintain the high level of DO_2 necessary to survive.

Shoemaker et al. (1973) considered that as patients who survived had higher cardiac index (CI), DO_2 and VO_2 than non-survivors, patients at risk should have these parameters optimised to the values obtained by the survivors.

He performed a study (Shoemaker et al. 1982) of first optimising fluid therapy to correct the underlying blood volume deficit. Then he directed therapy to not just restore normal values but to achieve optimal values. These optimal survivors values were,

- a) CI 50% greater than normal (4.5 L/min/m^2),
- b) DO_2 30% greater than normal (600 ml/min.m^2),
- c) VO_2 30% greater than normal (170 ml/min.m^2),
- d) blood volume 500 ml in excess of the norm (3.2 L/m^2 for males, 2.8 L/m^2 for females).

These increments supply the increased metabolic needs associated with fever and tissue repair. Severely traumatised and septic patients required much greater increments. This hypothesis was tested on 100 consecutive critically ill post-operative patients. Normal values were used as the therapeutic goals in the control patients, while the values obtained from the survivor group were used as goals in the protocol group. The mortality was significantly less in the protocol group (13%) than the control group

(48%). The number of life threatening complications was also greater in the control group.

It should be emphasised that this therapy of Shoemaker et al. (1982) has only been shown to prevent the complications of surgery of which ARDS and MSOF are included. It has not been shown to reverse the condition once it has started. But in preventing the establishment of ARDS and MSOF it does give an insight into the mechanism of the development of the syndrome.

They considered that five basic principles had been established;

- 1) Reduced or inadequate VO_2 leading to tissue hypoxemia was the primary pathogenic mechanism of the shock syndromes, providing a focus for the inflammatory response.
- 2) DO_2 , the supply side of the equation, limits VO_2 by either low flow as in haemorrhagic shock or maldistribution of flow, particularly at the microcirculatory level from inappropriate vasoconstriction or dilatation caused by neurohormonal mechanisms and the various inflammatory mediators, as occurs with trauma and sepsis.
- 3) There is an increased metabolic need (demand) in ARDS and MSOF patients that exceeds the basal transport function of the circulation (supply), that must be satisfied if the patient is not to suffer hypoxemia.
- 4) VO_2 represents the sum of all oxidative reactions and therefore reflects the body's metabolic activity; when VO_2 is not rate limited by blood flow, it is a measure of metabolic demand.
- 5) In reduced circulatory function, VO_2 is the regulatory mechanism; decreases in VO_2 stimulate the compensatory increases in DO_2 , heart rate, myocardial contractivity, cardiac output and ventilatory function, in an attempt to increase the VO_2 .

In essence the problem is a disparity between supply and demand of oxygen and the various nutrients transported by the circulation. The

common denominator in each of the shock syndromes associated with the development of ARDS or MSOF is reduced VO_2 . It is the earliest pathological event occurring at or before the initial hypotensive crisis signalling the shocked condition. Reduced or inadequate VO_2 is greater and more prolonged in patients who die than those who survive. Reduced or inadequate VO_2 is not just associated with development of shock, but the degree of VO_2 deficit is the major pathogenic factor in the development of shock syndrome and a major determinant of the outcome.

Oxygen diffusion.

The critical level of oxygen required for oxidative phosphorylation to proceed normally *in vivo* is unknown. Chance and Williams in 1955 showed in preparations of isolated mitochondria there was no significant decrease in the respiration of these organelles, as measured by the oxidation of cytochrome c, until the PO_2 in the perfusing media fell below 0.5 torr. In an isolated perfused lung preparation, the cellular PO_2 required to maintain aerobic function was studied by lowering the PO_2 in both the perfusate and the ventilating gas. A fall in ATP content and decrease in ATP/ADP was seen only when the PO_2 was reduced to 0.7 torr, a value not very different from the results seen in isolated mitochondria. This supports the concept that there is a very low resistance to oxygen diffusion within the cell. It is unlikely that the poor oxygen utilisation seen in ARDS and MSOF patients is due to problems of diffusion across the capillary and cell membrane.

Criticisms of the work on oxygen consumption in understanding MSOF.

A close examination of the work of Shibutani et al. (1983) does not show what was originally claimed. First of all the VO_2 value of 109 ml/min/m² is very low, the basal value during surgery previously reported by Guedel (Guedel 1924) was 138 ml/min/m². The work of Cain (1978), previously mentioned shows that an O_2ER of about 60% should be reached

before the critical point forces the VO_2 to fall. Calculating the O_2ER from the work of Shibutani et al. shows that the O_2ER was 24% when above the critical point and only 31% when below. Athletes can easily achieve O_2ER of 70%. Shibutani et al. (1983) patients appear to have an oxygen extraction problem and they do not have ARDS, sepsis or MSOF.

There is also a major difference between the work of Cain (1978) and that of Shibutani et al. (1983). Although they both demonstrate changes in DO_2 , these changes are achieved by very different methods. Shibutani et al. (1983) showed changes in DO_2 as a product of changes in the CO. Changes in the CO would change the blood flow to the various sites and the volume of blood to a particular organ. But most importantly it would be associated with a reduction in capillary density. Cain on the other hand, reduced DO_2 by producing hypoxemia, this reduced the volume of oxygen in the blood but maintained the CO. This had the effect of maintaining blood flow to the organs and within the organs. The number of capillaries perfused in Cain's work should remain the same after he produced hypoxemia. This would allow for a more efficient extraction of oxygen by maintaining the capillary density. This effect is seen by the high O_2ER in Cains work.

Shibutani et al. (1983) , measured the effect of differing CO, and saw the results of a redistribution of blood flow, both higher and lower in modifying the blood flow within an organ. When CO fell it caused a fall in capillary density and a reduction in O_2ER . This action would prevent an increase in O_2ER .

The principle criticism of the work presented on oxygen metabolism is that it is almost exclusively concerned with whole-body oxygen consumption. The relationship of blood flow, DO_2 and VO_2 in differing organs determines total blood flow, total DO_2 and total VO_2 . Flow independent organs such as the kidney, splanchnic bed, skin and muscle have low oxygen extraction rates. Whereas flow dependent organs such as heart and brain have very high oxygen utilisation. In principle, decreased

blood flow in flow independent organs does not reduce their oxygen consumption but reduced blood flow in a dependent organ will reduce its oxygen consumption. Although all organs can increase the capillary coefficient, so that they can extract more oxygen from that delivered, there is a critical point of about 60% O_2ER , over which, additional oxygen cannot be extracted.

Mitochondria

The reduction in O_2ER seen in patients with septic shock or ARDS and MSOF could be due to an inability of the mitochondria to utilise oxygen. Although oxygen is delivered to the tissue, a blockage in mitochondrial function might occur. Damage to the mitochondria, with the inevitable inhibition of mitochondrial respiration would result in a decrease in VO_2 regardless of DO_2 , similar to that seen in ARDS. The defect in mitochondrial function appears to correlate with the progressive systemic circulation failure seen in sepsis.

Endotoxin has been shown to have a variety of deleterious effects on isolated mitochondria, *in vitro*. These effects include the loss of respiratory control (Hift and Strawets 1961), the inhibition of succinate, glutamate, and malate oxidation (Lardy 1969), the inhibition of mitochondrial ATP-ase (Nicholas et al. 1972), the uncoupling of oxidative phosphorylation (Fonnesu 1960), and the deterioration of mitochondrial integrity (Hift and Strawets 1961).

The two mitochondrial reactions responsible for efficient ATP synthesis, the ATP-ase ATP synthetase reaction and the adenine nucleotide translocase function can be severely damaged in circulatory shock (Nicholas et al. 1972). The ATP-ase activity drops to about 30% of the control values 45 hours after the onset of haemorrhage or endotoxic injection. Adenine nucleotide translocase, an enzyme that transports adenine nucleotide across the inner mitochondrial membrane is inhibited by *in vivo*

endotoxin (Jones and Kramer 1978). A decrease in adenine nucleotide translocase activity could result in an accumulation of ATP inside the mitochondria which in turn would suppress the biosynthesis of ATP.

In haemorrhagic or endotoxic shock, the mitochondria of the liver, kidney and brain become functionally damaged at differing time intervals. Because of the extreme sensitivity of the brain to inadequate perfusion, brain mitochondrial metabolism is affected early in circulatory shock (Mellor et al. 1979, Baue and Said 1970).

Liver mitochondria, from rats, showed a reduction in ATP synthetic capacities (Mellor et al. 1971). Studies of liver mitochondria, from dogs, in endotoxic, septic or haemorrhagic shock have revealed severe deterioration of respiratory activity and structural integrity (Hift and Strawets 1961). These studies of hepatic mitochondrial energy functions in shock, from rats and dogs, indicate loose coupling of mitochondrial respiration after a delay of about one hour in haemorrhagic and two hours in endotoxic shock. Uncoupling of oxidative phosphorylation occurs, allowing oxygen to be consumed by hydrogen ions to produce water but no ATP. This effect is similar to that of the uncoupler, bromophenolbromine. The respiratory control ratios therefore drop progressively with time.

Structural studies of liver mitochondria performed by Hift and Strawets in 1961 have shown that lethal endotoxaemia and haemorrhage induce a heterogenous mix of hepatic mitochondrial changes ranging from grossly swollen profiles with loosened or disrupted matrices to forms with dense matrices and cristae. These are dilated but have normal external profiles. Although tissue hypoxia is present in shock, it alone is not responsible for the functional and structural changes of mitochondria in shock.

Changes of cardiac and skeletal muscle mitochondria in shock remain controversial. Some investigators have demonstrated normal mitochondrial respiration with adequate generation of ATP at the time of

cardiac failure (Mellor et al. 1974). After endotoxin induced cardiac failure, heart mitochondria do not show morphological damage, even in the presence of morphological evidence of inter-fibrilla swelling.

Similarly no deleterious alterations in the capacity of dog cardiac or skeletal muscle mitochondria to synthesise ATP were observed after endotoxaemia when liver and kidney mitochondrial functions were severely damaged. But in cardiac failure induced by endotoxaemia in the rat, impaired cardiac and skeletal muscle mitochondrial function and altered morphology was demonstrated by Schumer et al. (1971).

Unfortunately, mitochondrial alterations occur simultaneously with other sub-cellular defects caused by shock. No evidence exists to indicate whether the mitochondrial defects are primary or secondary to other intracellular alterations. Lysosomal enzymes released in shock have been shown to induce cell injury by Rangell et al. (1970).

Criticism of the concept of mitochondrial damage causing impaired oxygen uptake in shock was demonstrated by the work of Geller et al. (1986). They employed an endotoxic shock model in rats to determine the function of skeletal muscle mitochondria. No significant alterations in the parameters measured were observed in the endotoxic state. They also investigated bacterial peritonitis in the rat model in both liver and skeletal muscle mitochondria for possible alteration in liver metabolism. Neither muscle nor liver mitochondria exhibited functional impairment during sepsis and they concluded that neither endotoxaemia nor peritonitis selectively damages the mitochondria as had previously suggested. But this is only one paper among many opposing viewpoints.

Tissue oxygen utilisation.

When there is insufficient oxygen to sustain oxidative phosphorylation, increased rates of glycolysis provide for anaerobic production of ATP. Pyruvate acts as the proton acceptor from NADH₂ and

lactate is formed. If the dissociated proton from lactate exceeds the body's buffer capacity, metabolic acidosis will develop.

Lactate may either undergo oxidation or serve as a substrate for gluconeogenesis. Oxidation of lactate is accomplished through the tricarboxylic acid (TCA) cycle and is an oxygen dependant process. It takes place in the liver, renal cortex, heart and skeletal muscle. The rate of gluconeogenesis in the liver depends on hormones such as glucagon, insulin and glucocorticoids, in addition to the blood lactate level. The energy dependant process of gluconeogenesis also depends on the adequate oxygenation of the liver.

Tissue hypoxaemia is thought to be the most important cause of increased lactic acid level in the critically ill patient. When aerobic metabolism can no longer be maintained some tissues rely on glycolysis for energy production. Since this normally occurs only at very low oxygen levels, it represents very severe tissue hypoxaemia.

Plasma lactate levels are considered to reflect tissue hypoxemia and reduced or inadequate VO_2 . This concept is an over simplification, as plasma lactate levels are dependant on its production, clearance and excretion. The kidney excretes and clears lactate, but the greatest clearance is by the liver.

Recent work performed in pigs (Tighe et al. 1989) has shown plasma lactate levels in sepsis to be more dependant on liver blood flow and oxygen delivery to the liver, than the total body VO_2 . Septic pigs treated with the drug pentoxifylline showed similar whole body oxygen consumption as untreated pigs. Lactate production from the femoral muscle bed was similar in both groups of animals. But hepatic blood flow and oxygen delivery was significantly higher and arterial lactate significantly lower in the drug treated animals, than the untreated septic animals. Whole body VO_2 could not predict plasma lactate levels.

These studies strongly suggest that the rate of lactate clearance is a most important factor in the development of lactic acidosis in septic shock. Patients with septic shock who develop lactic acidosis may show a reduced hepatic lactate clearance rather than an inadequate VO_2 .

Additionally, Lang et al. (1983) have shown that in rats with faecal peritonitis, VO_2 rises by 40% at the same time as a 50% increase in plasma lactate occurs. They also presented histological evidence of liver leucostasis and congestion as well as renal congestion. This again suggests that inadequate clearance of lactate is the cause of the high serum lactic acid.

According to the work of Cain (personal communication), VO_2 can rise at the same time as blood lactate levels in dogs infused with endotoxin. Pyruvate levels also rise. This is considered to be due to the inactivation of pyruvate dehydrogenase, preventing the entry of pyruvate into the mitochondria by stopping the formation of acetyl co-A. Pyruvate dehydrogenase exists in two forms, the active and inactive. Sepsis or endotoxin can cause the conversion of active PDH into inactive PDH.

Phosphocreatine/ DO_2 ratio

Energy production can be divided into aerobic metabolism producing 36 molecules of ATP per molecule of glucose through oxidative phosphorylation, or anaerobic metabolism producing 2 molecules of ATP per molecule of glucose through glycolysis, the creatine kinase (CPK) reaction or the adenylate kinase reaction. Glycolysis increases cytosolic hydrogen ion concentration reducing the intracellular pH. Consequently, glycolysis is not the preferred source of ATP during hypoxemia.

ATP formed in the mitochondria is freely available to the cell by diffusion across the mitochondrial membrane or by the action of phosphocreatine (PCr) energy shuttle. The ATP molecule is large and diffuses into the cytoplasm with difficulty, therefore it is thought to stay within the mitochondria. The PCr molecule is quite small and freely

diffuses within the cytoplasm. According to the PCR-energy shuttle hypothesis, ATP phosphorylates PCr in the mitochondrial membrane by the creatine kinase reaction. PCr carries the high energy phosphate from the mitochondria to the various energy consuming portions of the cell, where it phosphorylates ADP to form ATP and creatine. The PCr reaction also has the advantage of consuming hydrogen ions during the formation of ATP. The creatine kinase reaction has been shown to occur in many tissues, but the highest concentration of creatine kinase is found in the tissues of high energy requirements, the heart, skeletal muscle and brain. But the beneficial effects of this reaction are short-lived because of limited tissue concentrations of PCr. The adenylate kinase reaction helps maintain ATP by splitting 2 ADP in to one AMP and one ATP.

adenylate kinase



AMP diffuses from the cell into the microvasculature where it binds to receptors on blood vessels to cause potent vasodilatation.

Oxygen delivery and consumption are the two parameters judged to be of clinical importance in measuring the adequacy of tissue oxygenation. Nuclear magnetic resonance studies on cellular metabolism reveal how insensitive, and at times misleading, these indices can be. Measurement of tissue ATP, phosphocreatine, and inorganic phosphate (Pi) can correlate with DO₂, VO₂, or lactate, or all of them (Clark et. al. 1987)

Studies using magnetic resonance spectroscopy (MRS) in hypoxic rat heart preparations revealed that ATP concentrations were maintained by depleting high energy PCr stores. There is an accumulation of Pi, with depletion of PCr stores after 5 minutes ischemia and a gradual decrease of ATP levels. Similar responses have also been found in brain and heart by Sapega et al. (1985).

The decrease in PCr and the increase in Pi provide a convenient index of tissue hypoxemia: the PCr/Pi ratio. In fully aerobic tissue, the

PCr/Pi is high, about 7-10. With hypoxemia, it declines progressively to levels less than 1, and further decreases result in a fall of ATP.

Re-perfusion after 3 hours of ischaemia resulted in a rapid and complete reversal of these changes. Phosphocreatine resynthesis was detected within 10 seconds after reperfusion and complete recovery required approximately 1 minute for every preceding hour of ischaemia. The oxygen debt did not have to be repaid. It should be emphasised that these studies were not done in septic animals and the findings may not be applicable to humans suffering from ARDS.

The work of Idstrom et al. (1985) represents the only study published where changes in cellular bioenergetics correlated with changes in DO_2 and VO_2 . Idstrom et al. (1985) found a linear correlation in the PCr/Pi ratio with DO_2 and VO_2 in a rat hind limb preparation. When VO_2 began to decline as a function of DO_2 , ATP turnover rate was maintained constant at the expense of energy stores in the form of phosphocreatine and the use of anaerobic metabolism.

The studies of Idstrom et al. (1985) involved perfusing the rat hindlimb with Krebs-Henseleit bicarbonate buffer without erythrocytes. The perfusate was equilibrated with gas containing 5% CO_2 and fractions of oxygen varying from 95% to 20% with the remainder nitrogen and the preparation perfused at a constant flow. P^{31} -MRS spectra were obtained from the rat leg at different levels of DO_2 , with the limb at rest, during stimulation and finally during recovery. The relationship between DO_2 and VO_2 obtained during these experiments was a linear function. Likewise, the authors found a linear relationship between the PCr/Pi ratio and DO_2 .

Gutierrez and Pohil (1989) performed experiments on the isolated hindquarter of rabbits with normal DO_2 , hypoxemia and reperfusion. The data showed that there were 2 distinct groups, group A with a biphasic DO_2 - VO_2 relationship and group B with a linear relationship having a higher

resting VO_2 . The reason for the spontaneous development of two differing groups remains unclear.

Throughout the experimental procedures in both groups ATP concentrations were maintained at near control levels. During hypoxemia the $\text{DO}_2\text{-PCr/Pi}$ in group A showed a biphasic relationship similar to that of $\text{DO}_2\text{-VO}_2$, seen in normal subjects. In group B, the $\text{DO}_2\text{-PCr/Pi}$ association was nearly linear, as DO_2 fell so did PCr/Pi .

After the hypoxic phase, some animals showed that during reoxygenation, despite a return of DO_2 and VO_2 to control level, the PCr/Pi ratio remained low in both groups, and did not return to control levels during the allotted time for the recovery phase of the study. When the perfusion was continued past the recovery time, almost invariably there resulted a relentless fall in ATP, with a decrease in VO_2 . These events signaled irreversible cellular damage. Although this phenomenon remains unexplained, it appears likely that irreversible cellular damage occurred during hypoxemia.

Control lactate concentration in group A was always lower than Group B; when DO_2 fell, there was only a small rise in plasma lactate in group A, but a large rise in group B.

Group A had a lower control O_2ER corresponding to a lower resting VO_2 . These animals were able to increase their O_2ER from a control value of 20% to approximately 60%. Additionally by increasing the O_2ER only to 40%, they could maintain control VO_2 at half the control DO_2 . The lower control VO_2 and the ability of the microcirculation to triple the O_2ER allow group A to maintain their control level of metabolic activity by generating ATP exclusively from oxidative phosphorylation. This results in a constant VO_2 , with a reduced DO_2 , preservation of the PCr stores and avoidance of anaerobic glycolysis as a source of ATP. This is confirmed by the stable plasma lactate concentration. When the extraction limits were reached, VO_2 began to decline and ATP was generated via PCr .

The group B, animals had difficulty increasing the fraction of oxygen extracted from capillary blood. They had a high control O_2ER of nearly 40%, giving the animals a disadvantage as the maximum O_2ER for skeletal muscle is 60%. Consequently these animals could not increase their O_2ER sufficiently to supply their greater ATP requirements. When DO_2 began to decrease, VO_2 also fell. Oxidative phosphorylation could no longer satisfy tissue requirements for ATP and the PCr stores were used, the PCr/Pi ratio declined with the VO_2 . When PCr was nearly depleted, with a PCr/Pi ratio of nearly 1, the lactate levels started to rise.

It must be assumed that the apparent inability to increase the O_2ER in the face of a falling DO_2 , so lethal to the outcome of the illness, is due to the inappropriate perfusion of supply independent organs and the failure to recruit sufficient capillaries in an organ. The reason why this inappropriate lethal response occurs remains to be investigated.

ATP Levels in sepsis.

Chaudry et al. (1979) investigated the concentrations of ATP, AMP, lactic acid, and the hepatic blood flow in rats with faecal peritonitis over 18 hours, shown in Table 32. The rats were divided into 3 groups, sham operated served as controls, "early sepsis" killed after 10 hours and "late sepsis" killed after 18 hours. Tissue was taken from the kidney, liver, gastrocnemius muscle (intermittently working muscle) and the diaphragm (constantly working muscle). Values in the early sepsis group were similar to the sham group.

ADP is the degradation product of ATP and should rise as the latter falls. But when hypoxemia deprives the cell of oxygen, ATP can be produced in some organs by two molecules of ADP forming one molecule of ATP and one AMP. In these organs the cellular AMP concentration will rise, when the relevant enzyme is not present, the cellular concentration of AMP will remain stable. Pyruvate is converted to lactate for glycolysis to proceed, so

that lactate is an indicator of anaerobic metabolism. An equal lactate to pyruvate ratio indicates that pyruvate cannot enter the mitochondria and is converted to lactate as its concentration rises. If the lactate level rises without an increase in pyruvate then it suggests that there is an inadequate supply of oxygen preventing the oxidative phosphorylation process from proceeding, (NADH converts pyruvate to lactate in the absence of oxygen).

Table 32. Metabolic changes in the various tissues of rats with faecal peritonitis. (Chaudry et al. 1979)				
	Liver	Kidney	Gastro	Diaphragm
ATP				
Sham	1.99	1.23	5.07	3.39
Late septic	0.71*	0.77*	5.10	3.35
AMP				
Sham	0.50	0.64	0.06	0.14
Late septic	1.00*	0.64	0.04	0.12
Pyruvate				
Sham	0.17	0.23	0.18	
Late septic	0.12	0.10*	0.21	
Lactate				
Sham	1.79	2.45	4.75	
Late septic	5.29*	5.12*	9.15*	
Lac/Pyr Ratio				
Sham	10.5	10.6	26.3	
Late septic	44.0*	51.2*	43.5*	
Blood flow				
Sham	23.9			
Late sepsis	15.5*			

* P< 0.001 compared with sham operated animals

In late sepsis, liver ATP fell by 65% and blood flow decreased, AMP and the lactate to pyruvate ratio increased. This conversion of ADP to ATP, and lactate production suggests effective cellular hypoxemia. Renal ATP fell by 45%, a smaller amount than in the liver. But unlike liver AMP, which rose, renal AMP level did not change, showing that it cannot convert ADP to ATP. Also, the pyruvate concentration fell and lactate production rose with a bigger increase in the lactate to pyruvate ratio than liver, indicating that unlike the liver, glycolysis was developing in kidney to maintain ATP levels.

The change in diaphragm and gastrocnemius muscle differ markedly from those of the liver and kidney. The ATP and pyruvate levels showed no change. But lactate and the lactate/ pyruvate ratio showed a considerable increase in anaerobic metabolism to maintain ATP levels.

Although increases in anaerobic metabolism would be expected to be associated with falling levels of ATP, this did not occur. The maintenance of ATP is probably due to the highly active glycolytic system of skeletal muscle and their mobilisation of high energy phosphocreatine to resist effects of reduced blood flow and hypoxemia.

There is a decrease in the energy available to liver and kidney but not skeletal muscle during late sepsis. This could reflect a more critical fall in blood flow to the liver and kidney or the metabolic differences of the various organs.

A further marker of inadequate tissue oxygenation involves the degradative pathway of ATP. If ATP utilisation exceeds its production, ADP or AMP accumulates and is hydrolysed through a series of steps to hypoxanthine. This substance can cross the cell membrane and then be further degraded by oxidation in the liver via the enzyme xanthine oxidase to xanthine and then uric acid. It has been shown that an elevation of hypoxanthine correlates to decreased survival in ITU patients (Grum et al. 1985).

In the septic stage there may be an inhibition of skeletal muscle's ability to oxidise glucose effectively. This abnormality relates to a metabolic block of the entry of pyruvate obtained from glucose in the Krebs cycle. Pyruvate dehydrogenase can become inactive in septic subjects. As a result pyruvate tends to accumulate which in turn equilibrates at increased levels with lactate. Lactate levels become progressively higher as the septic process accelerates. This is the cause of lactic acidemia of sepsis and is accompanied by a normal lactate pyruvate ratio in contradistinction to states associated with a perfusion deficit.

An important feature of this work is the difference in the ability of diverse organs to maintain ATP production. Liver suffers principally from hypoxemia, more so than kidney and muscle, a possible reason for the great increase in splanchnic blood flow showed in high risk patients. It also demonstrates the vital importance of maintaining hepatic DO_2 . A study of the relationship of ATP production in various organs to the increase in DO_2 and VO_2 in those patients treated with high DO_2 would be of considerable importance.

Chapter 4

Histological features associated with M.S.O.F.

Introduction

Investigation of the ultra-structure of the lungs was undertaken to establish the mechanisms involved in the development of this condition. Studies were performed on lungs of recently deceased patients, but unfortunately their condition did not reflect the mechanisms involved, only the degenerative changes that occurred before death. Considerable confusion has arisen because of these early studies, cause being mistaken for effect.

Bachofen and Wiebel in 1977 and Schnells et al. in 1980 were the first to perform open lung biopsies on ARDS patients as early as 3 hours after the onset of symptoms. From these studies they were able to describe some of the early changes and postulate the causes.

Unfortunately, histological studies of human tissue from patients with multi-organ damage do not allow the study of the development of the condition. Animal models have been developed to mimic the organ changes seen in humans. The relevance of these studies must be questioned in view of the considerable differences between humans with sepsis and the experimental septic animal. The latter is a relatively healthy and intact organism which can sustain a massive insult (large doses of live organism or endotoxin) and is followed for only a number of hours, rarely a few days. Whereas a patient may have had the condition for several days and is treated aggressively with therapies that are untested.

Considerable work has been done in the non-human primate (baboon) as this animal's response to sepsis closely resembles that of the human. Unfortunately, the baboon is expensive to study, carries hepatitis and can be distasteful to work with on ethical grounds due to its primate status.

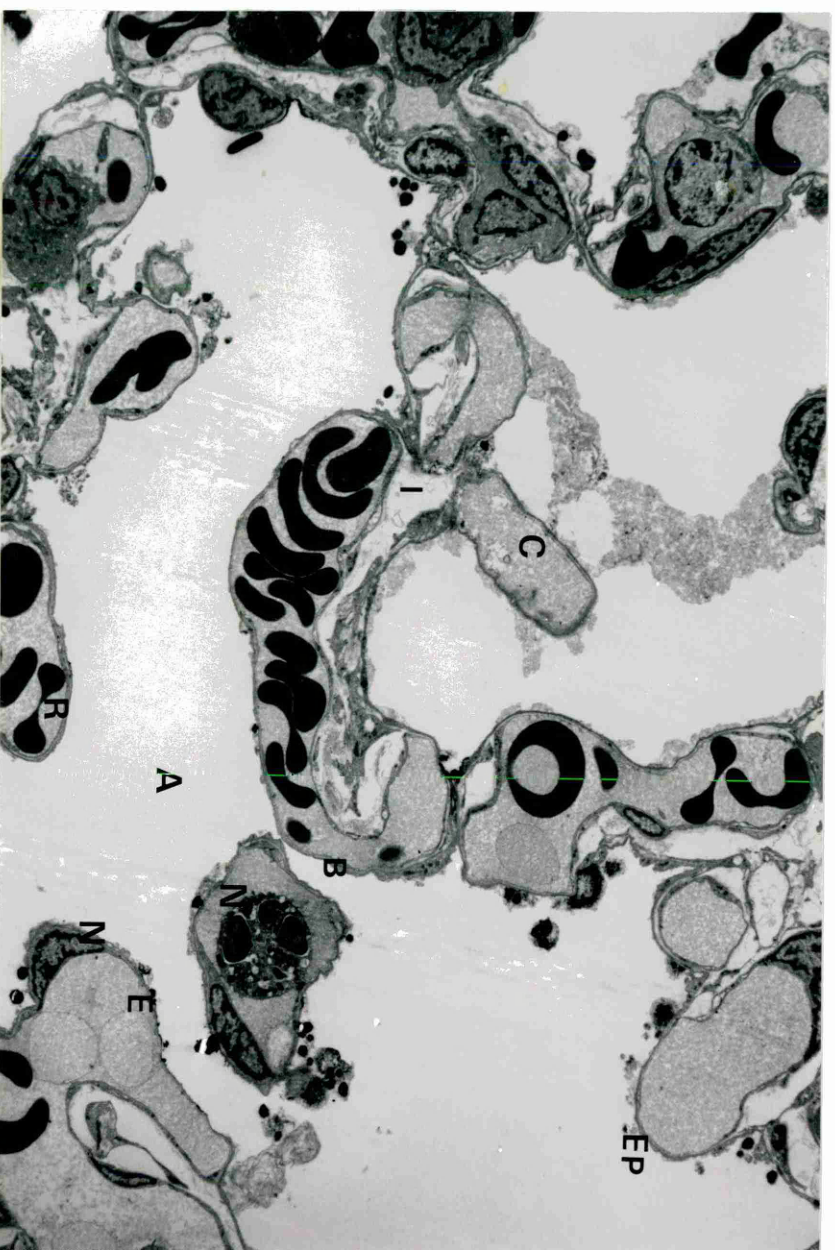


Figure 2. Lung section from a pig taken before infection.

Normal thin walled pulmonary capillaries (C) can be seen, containing the flattened nuclei (N) of endothelial (E) cells. They are in alveolar (A) sacs that are lined with epithelial (EP) cells. The capillaries contain biconcave red blood cells (R) and neutrophils (N). Between the endothelial and epithelial cells can be seen the basement membrane (B). The interstitium (I) can also be seen. (TEM X 1000)



Figure 3. Neutrophil in a pig pulmonary capillary, sampled before infection. The three layers of the capillary wall can be seen, alveolar epithelium (EP), basement membrane (BM) and capillary endothelium (E). The azurophilic (A) and specific granules (S) are membrane bound and can be easily distinguished. (TEM X 8500)

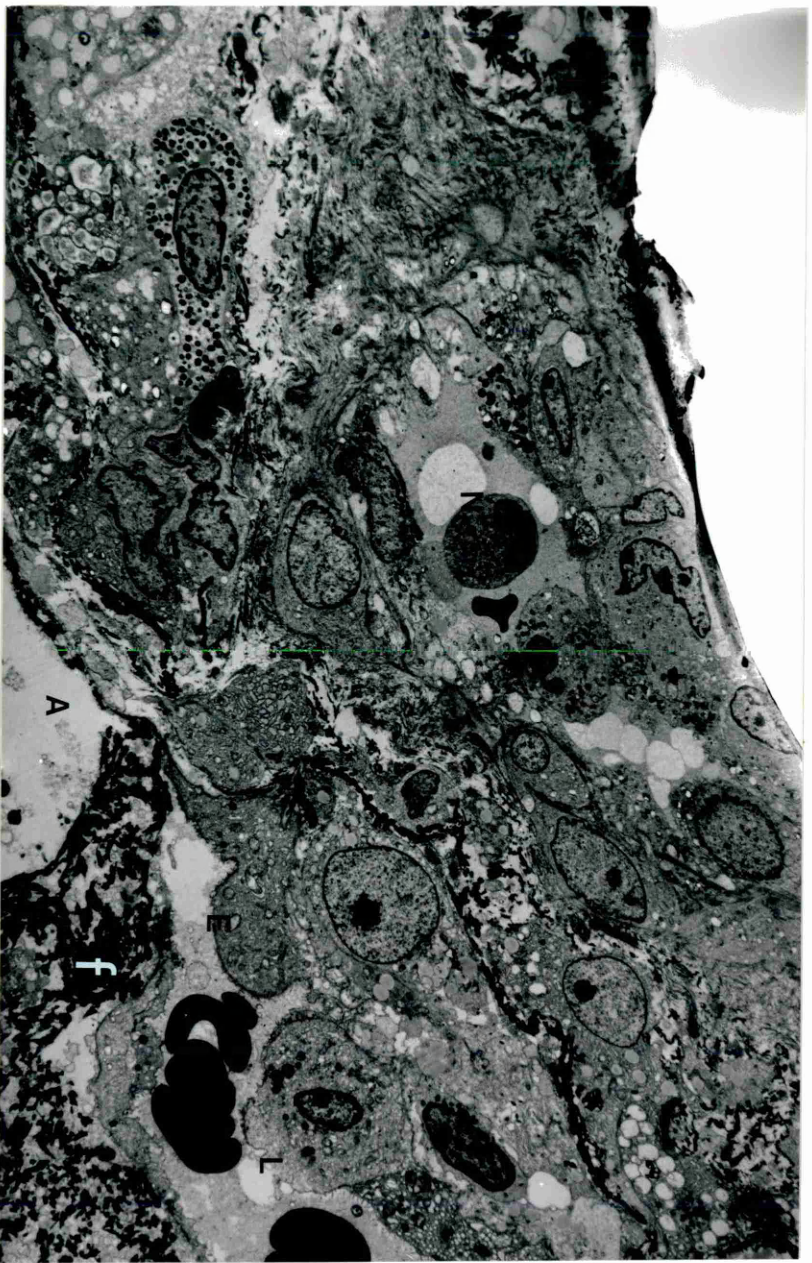


Figure 4. Lung sample from a patient with ARDS. It shows total derangement of pulmonary architecture. The alveolar (A) space is lined with fibrin (F), and the endothelium (E) is swollen. There is considerable infiltration by both neutrophils (N) and lymphocytes (L). (TEM X11550)

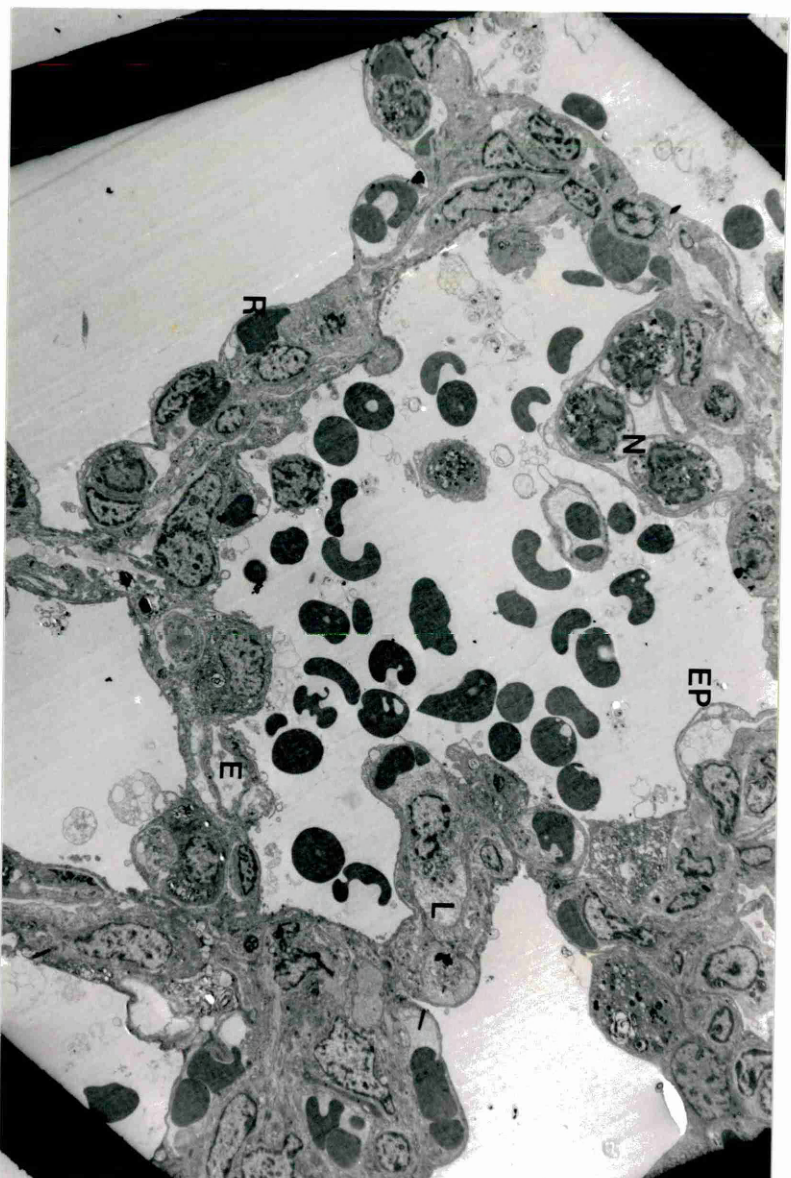


Figure 6. Pig lung after 5 hours of infection. Capillary patency is reduced, by degranulating neutrophils (N), lymphocytes (L), swollen red blood cells (R), hypertrophied endothelium (E) and cell debris. Alveolar epithelium (EP) is swollen and vacuolated. Red blood cells and leucocytes in the alveolar space demonstrate capillary rupture. (TEM X1000)

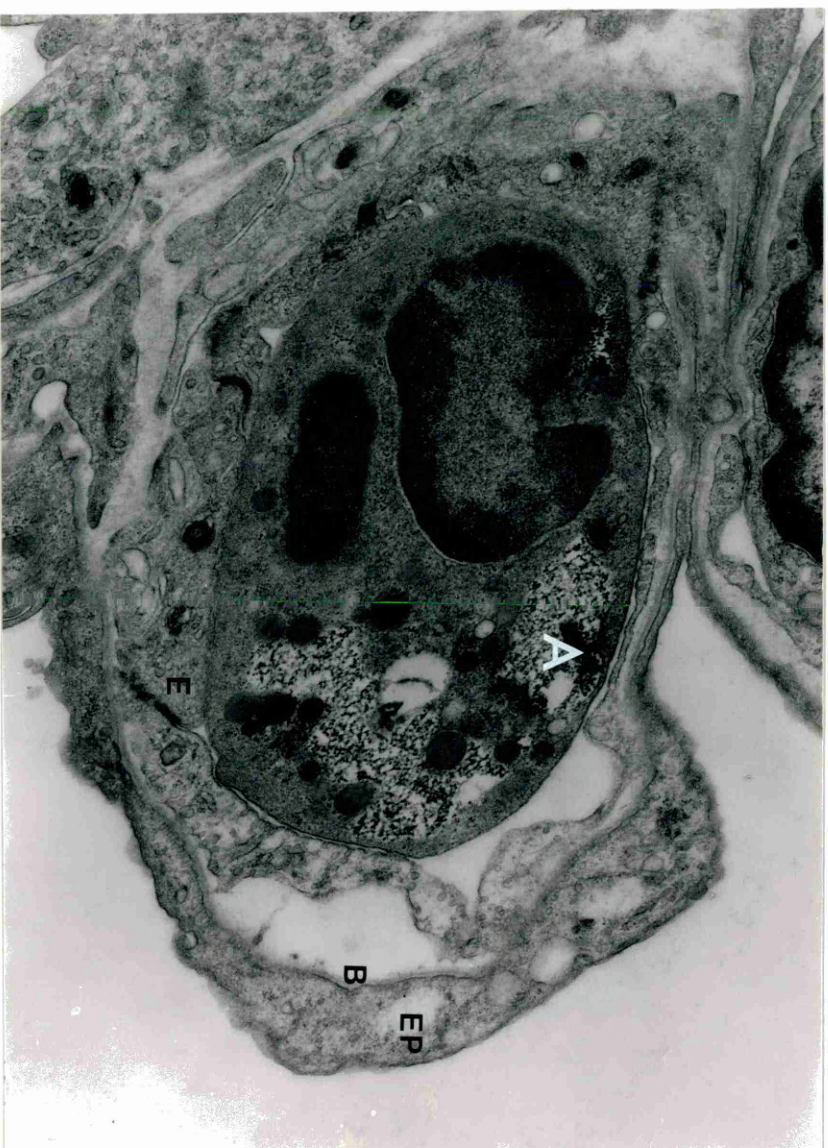


Figure 7. Neutrophil in a pig pulmonary capillary after 5 hours of infection. It shows a degranulating neutrophil. The azurophilic granules (A) are not membrane bound. The neutrophil appears to be adherent to the endothelium (E), which is swollen and partly detached from the basement membrane (B). The epithelium (EP) is swollen and vacuolated. (TEM X 8500)



Figure 8. Pig pulmonary capillary after 5 hours of infection. The pulmonary capillary wall can be seen with contact points between the neutrophil (N) and endothelium (E). (SEM X 6700)

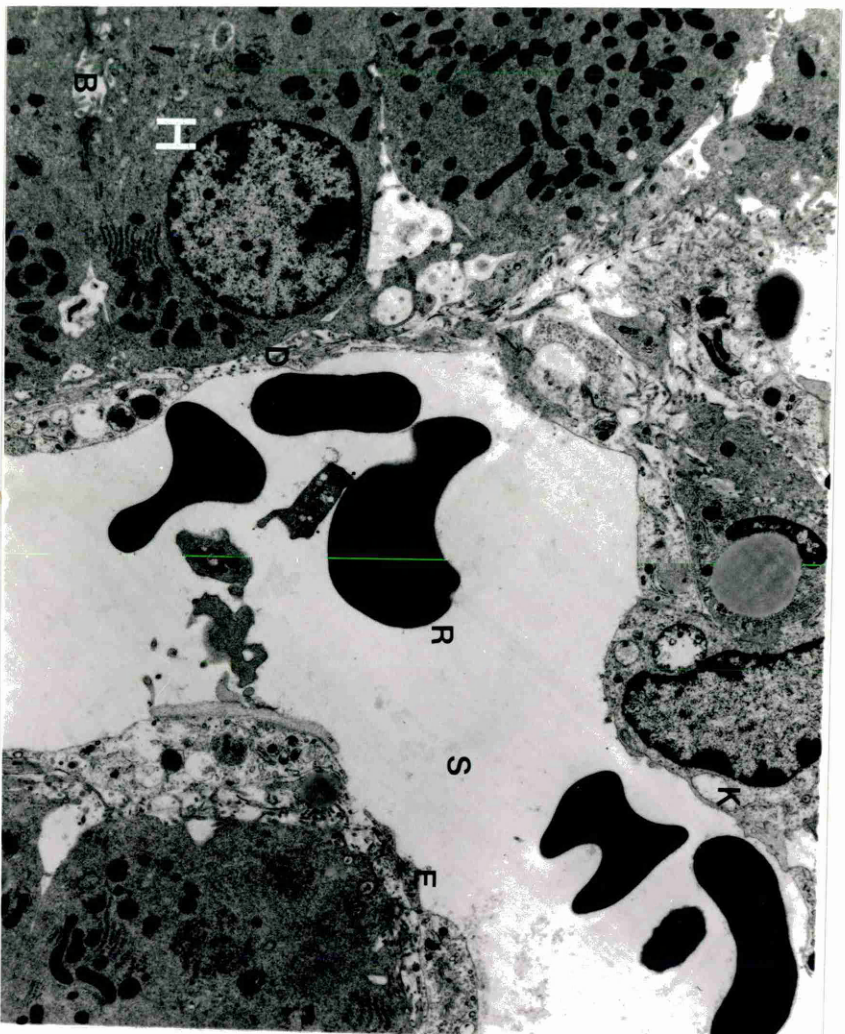


Figure 9. Sinusoid of pig liver section taken before infection.
The sinusoid (S) containing red blood cells (R) and a Kupfer cell. (K). It is lined with endothelium (E), covering the space of Disse (D). Hepatocytes (H) and bile canaliculi (B) can be seen. (TEM X 3000)

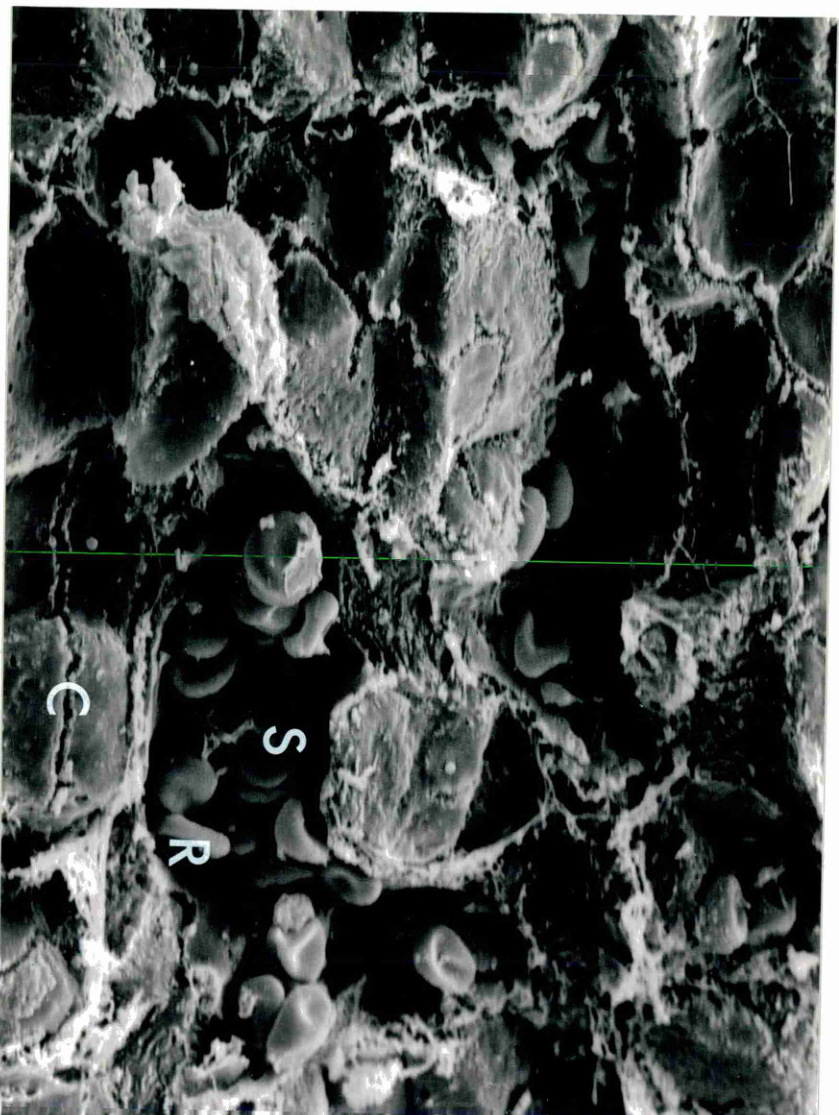


Figure 10. Pig liver section taken before infection. The normal hepatic architecture is clearly defined, with open sinusoids (S) containing biconcave red blood cells (R). The bile canaliculi (C) are clearly defined. (SEM X1780)



Figure 11. Sinusoid of pig liver section taken after 5 hours of infection. The sinusoid is occluded by degenerating neutrophils (N) containing bacteria (B), lymphocytes (L), cell debris and fibrin deposits (F). Kupfer (K) and endothelial (E) cells show hypertrophy with obliteration of the space of Disse. The hepatocytes contain lipid droplets (L). (TEM X4000)



Figure 12. Pig liver section taken after 5 hours of infection. The sinusoids are occluded with indeterminate cell debris and the bile canaliculi (B) are not clearly defined. (SEM X 1780)



Figure 13. Sinusoidal endothelium from a pig liver section taken before infection. Part of a sinusoid lined with fenestrated endothelium (E). The space of Disse (D) can be seen through the fenestrations. (SEM X 6200)

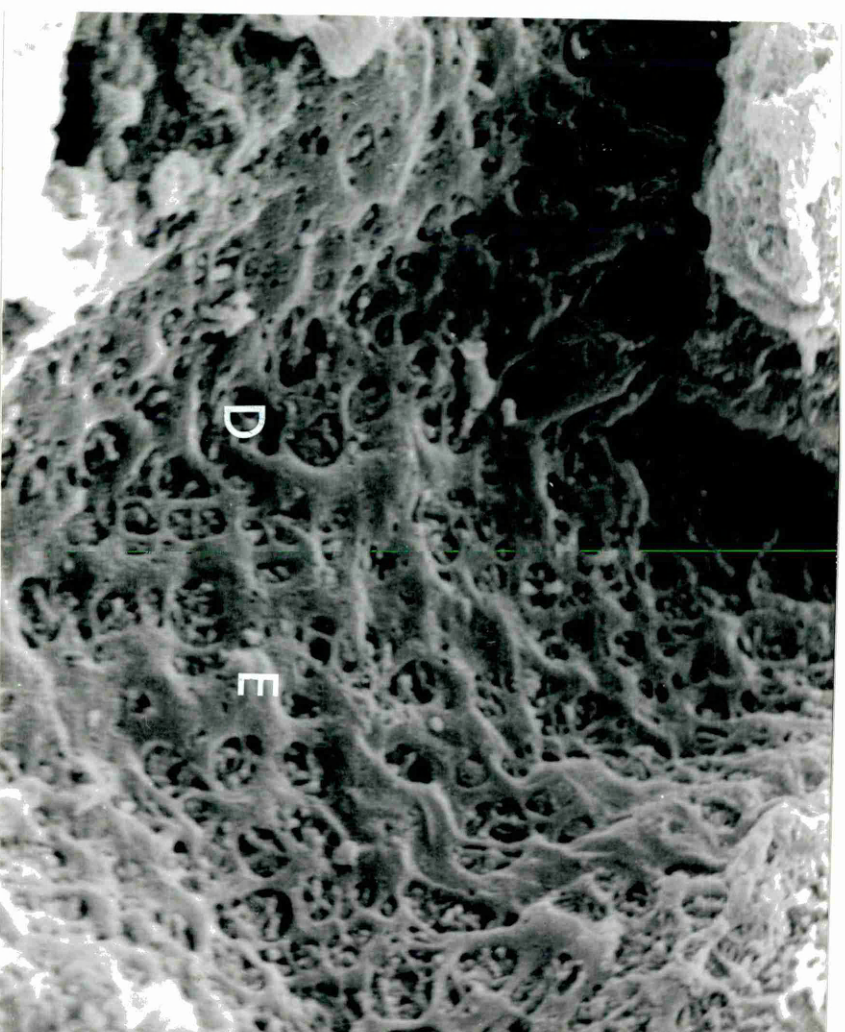


Figure 14. Sinusoidal endothelium from a pig liver section taken after 5 hours of infection. The space of Disse (D) can be seen through the sieve plates of the fenestrated sinusoidal endothelium (E). This endothelium can be seen to be disrupted by areas of swelling and larger fenestrations. (SEM X 6200)

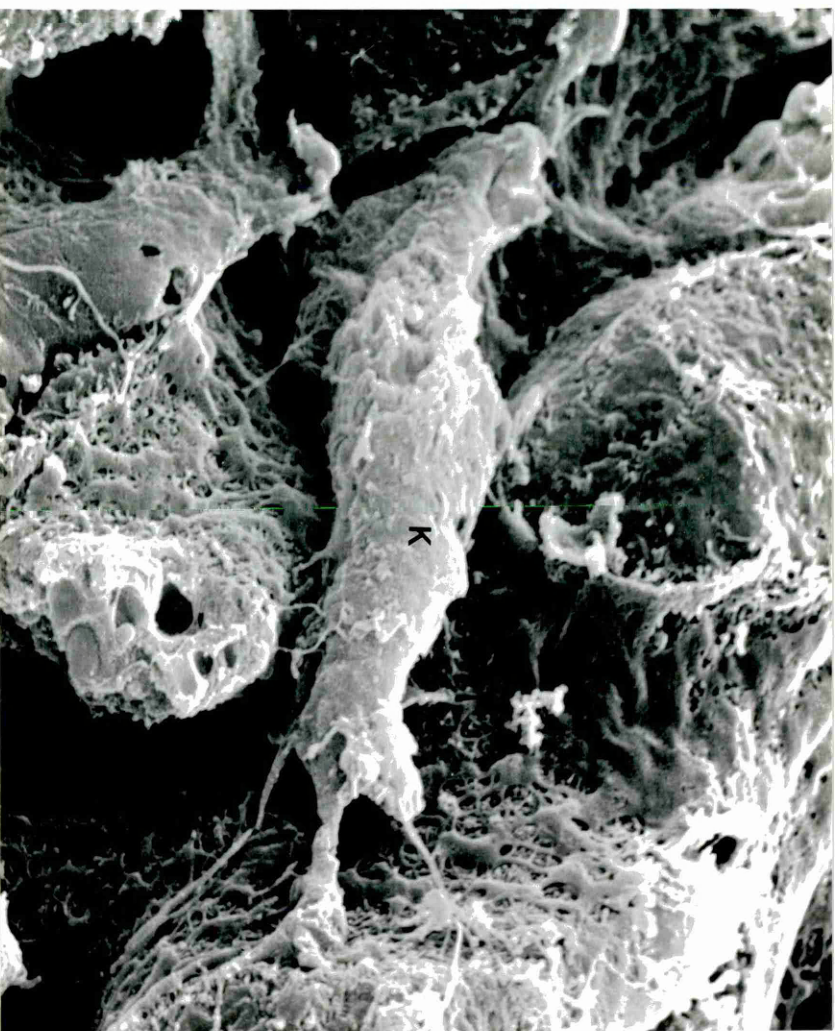


Figure 15. A swollen Kupffer cell from a pig liver section taken after 5 hours of infection. Obstruction of a sinusoidal bifurcation can be seen by the swollen Kupffer cell (K), with swollen fenestrations of the endothelium immediately adjacent to it but not further away. (SEM X 3000)

Lung Histology

Normal pulmonary ultrastructure

On the gaseous side of the normal alveolar wall are two main cell types: epithelium and type II pneumocytes. Epithelium covers 90% of the alveolar surface and are a layer of flattened cells with thin cytoplasmic extensions less than 1 μm thick. Junctions between alveolar cells are very tight and impermeable to water. When damaged, epithelial cells cannot replicate. They are formed by differentiating type II pneumocytes as described by Adamson and Bowden (1975). Type II cells have numerous functions, the most important is the production of surfactant, maintaining the alveolar sacs open.

The work of Mason (1978) shows that although type II pneumocytes develop into epithelial cells, under adverse conditions they may remain as large cuboidal cells. These cells appear relatively inactive in secreting surfactant from their lamellar bodies or cytoplasmic microsomes, and their thickness prevents oxygen exchange.

Lining the pulmonary capillaries are the endothelial cells shown (Mason et al. 1978) to be functionally important, containing large numbers of enzymes capable of degrading prostaglandins and vasoactive amines or producing prostaglandins. Endothelium is also responsible for the conversion of angiotensin 1 to 2 or the inactivation of bradykinin.

The normal ultrastructure of pig lung is shown in the transmission electron micrograph (TEM) in Figure 2. Red blood cells and several leucocytes are contained within the thin walled pulmonary capillaries. Nuclei of endothelial cells are flattened inside the capillary. A normal neutrophil in a pulmonary capillary can be seen in the TEM in Figure 3. Its azurophilic and specific granules are membrane bound and can be distinguished from each other. The three layers of the capillary wall can be distinguished, on the alveolar side is the epithelium, in the middle the basement membrane and inside is the capillary endothelium.

Pathological pulmonary ultrastructure

There are several reviews of the electron microscopic features of the lung in humans who developed ARDS after trauma (Schnells et al. 1980) (Pietra et al. 1981), burns (Nash et al. 1974), septicaemia (Bachofen and Weibel 1982), and pancreatitis (Bachofen and Weibel 1977, 1974). Although these conditions are of considerable difference, the pulmonary changes are essentially the same.

In humans, two phases develop between the onset of the condition and the 21 days that often pass before the death of the patient, the acute and chronic phases.

The description of Bachofen and Wiebel (1982) is typical of many of the reviews. They studied lung tissue from 9 patients dying from ARDS due to septicaemia. In the acute phase, up to 4 days after the onset of respiratory distress, the alveolae contained erythrocytes, neutrophils, macrophages, oedema fluid and strands of fibrin. Epithelium was ruptured with exposure of the basement membrane. Twenty-four hours after the onset of symptoms, type II pneumocytes proliferated within the alveolar lumina. Hyaline membranes comprising a mixture of plasma proteins, fibrin strands, cell debris and degenerative changes in elastic fibres were also seen.

There was irregular endothelial cell swelling, vacuolation and exposure of basement membrane. The initial injury was to the endothelial cells, by aggregates of lymphocytes and degranulating neutrophils (Siegel and Cerra 1979). Associated with this development was fibrin deposition in the pulmonary lumina and the formation of microthrombi.

Pulmonary oedema either interstitial or intra-alveolar was reported in this condition. Intra-alveolar oedema was seen as a terminal event of ARDS. The excess fluid in the interstitial space is removed by the pulmonary lymphatics (Hurley 1978, Cunningham and Hurley 1972) but when the capacity of the lymphatics is exceeded, oedema spreads into the interstitium and the alveolar lumina.

Not all patients necessarily follow this sequence of diffuse alveolar damage, progressing to interstitial and intra-alveolar fibrosis (Bachofen and Weibel 1974). Bachofen and Weibel in 1977, quantified the components of the alveolar wall in ARDS due to septicaemia. They showed approximately a twofold increase in the interstitial volume in the acute phase and up to 10 times normal in the chronic phase. Similarly there was a two to threefold volume increase in the epithelial cells in the acute phase and a further doubling in the chronic phase. Both these changes impair gaseous diffusion with the smallest relative volume changes being those of the endothelial cell.

Schnells et al. (1980), took lung biopsies from patients during the course of post-traumatic respiratory insufficiency between 3 hours and 19 days after the onset of shock. There was endothelial and epithelial damage, lymphatic dilatation and interstitial as well as intra-alveolar fibrin extravasion. Interstitial oedema and extra vascular migration of granulocytes, lymphocytes and mast cells with fibrinoblastic proliferation and fibrosis of the alveolar septa was also seen. Fibroblasts replaced capillaries at the surface where gas exchange takes place, the capillaries were thus forced into the depth of the interstitial tissues. At the same time the alveolar surface became covered with layers of thick walled proliferating epithelia which also render gas exchange increasingly difficult.

Figures 4 and 5 are TEM of lung tissue, removed within minutes of the death of a patient with ARDS. Figure 4 shows the total destruction of the normal pulmonary architecture. It is difficult to establish where gas exchange was taking place. The alveolar space is lined with fibrin and the normal structures are totally deranged. Figure 5 shows a single pulmonary capillary obstructed by a degranulating neutrophil. The endothelium is swollen and interstitial oedema can be seen. The alveolar epithelium is separated from the basement membrane which is covered by fibrin.

Animal studies of pulmonary ultrastructure in ARDS like syndrome.

We have studied rabbit (Tighe et al. 1987, 1989) and pig (Tighe et al. 1990) pulmonary ultrastructure from lung biopsies taken 2 and 5 hours after the induction of faecal peritonitis. Two hours after the induction of peritonitis there was an increase in the number of neutrophils in the pulmonary capillaries, but without apparent damage. After 5 hours of peritonitis there was an eightfold increase in the number of neutrophils and lymphocytes. The neutrophils were degranulated and the capillaries occluded, with very few red blood cells (RBC). The capillary endothelium was either swollen, vacuolated or completely disrupted, exposing the basement membrane. The alveolar epithelium is vacuolated or detached from the basement membrane. Type II pneumocytes did not show the presence of lamellar bodies, suggesting the absence of surfactant production.

We demonstrated (Tighe et al. 1990) that in pig lung 5 hours after the induction of faecal peritonitis, capillary luminal area was much smaller than normal tissue and there was an 8 fold increase in the number of intracapillary leucocytes (Tighe et al. 1990). The patent perfusable capillary area was reduced and partially occluded by degranulating neutrophils, lymphocytes, swollen RBC's, enlarged endothelium and cell debris. The alveolar epithelium is swollen and vacuolated. Some capillaries were ruptured as evidenced by the RBC's and leucocytes in the alveolar space seen in Figure 6. Figure 7 shows a capillary occluded by a degranulating neutrophil, appearing to be tightly adherent to the endothelium, which is swollen and partly detached from the basement membrane. The epithelium is swollen and vacuolated. Figure 8 is a scanning electron micrograph (SEM) of a pulmonary capillary containing a neutrophil. Points of adhesion can be seen between the neutrophil and the endothelium.

Meyrick et al. (1983) infused *E. coli* endotoxin into 5 sheep and compared them to a control group. After 50 minutes of endotoxin infusion,

light microscopy revealed margination and accumulation of leucocytes in the lung microcirculation. Counts of the number of granulocytes in lung biopsy specimens from test animals showed a threefold increase by 15 minutes and a sixfold increase by 240 minutes. Electron microscopy of the leucocytes identified both granulocytes and lymphocytes in approximately equal numbers. Some granulocytes were fragmented and specific granules were found free in the vascular lumen. By 60 minutes interstitial oedema was seen and there was focal endothelial damage. Correlation of the structural and physiological changes showed that the initial accumulation of leucocytes in the microcirculation occurred when pulmonary hypertension developed. The migration of leucocytes into the interstitium and the endothelial cell damage preceded the physiological changes that were interpreted as increased pulmonary capillary permeability.

Studies of anaesthetised baboons (Coalson et al. 1977) infused slowly with live *E. coli* bacteria demonstrated sequestration, degranulation and fragmentation of neutrophils and platelets in the pulmonary capillaries. There was characteristic swelling and disruption of the pulmonary endothelium. Fibrin was present in the capillary lumen, interstitium and alveolar sac and oedema was also present in the interstitium and alveolar sac.

Shaw et al. (1980) made intra-tracheal injections of C5a in rabbits to induce an acute pulmonary inflammation characterised by intra-alveolar accumulation of neutrophils, erythrocytes and oedema fluid. Electron microscopy of the rabbit lung revealed degranulated neutrophils in the pulmonary capillaries and interstitial spaces. The alveolar spaces, and less often, the interstitial compartment contained fibrin deposits with leucocytes and erythrocytes enmeshed in the fibrin strands. Injury to the pulmonary endothelium consisted of bleb formation and exposure and separation of the basement membrane, with accumulation of inflammatory cells in the capillaries. Epithelial cells were damaged with blebbing and detachment

from the basement membrane. Endothelial and epithelial cell damage was always associated with pulmonary neutrophils continuous with the injured structure.

Balis et al. (1978) infused *E. coli* endotoxin into baboons for up to 22 hours, and found a rapid margination of neutrophils in the pulmonary capillaries that progressively degranulated and fragmented. The capillary endothelium was swollen and disrupted. There was interstitial and alveolar oedema. The alveolar epithelial cells showed blebbing and disruption with exposure of the basement membrane.

Attention has been paid to histological changes in the lung of humans with ARDS, and lung and liver in animal models of this condition. They are considered to give a good indication of the mechanisms causing MSOF. But the pathophysiology of this condition is undoubtedly much more complex. The work of Beeley et al. (1986) is especially illuminating.

They took 36 rabbits divided into 3 groups and allowed to inhale acrolein vapour for 15 minutes. After 30 minutes animals from two of the groups received steroid treatment and the other group was given a placebo. Some animals died during the study, others were killed after 72 hours. Histology of the lungs was examined in all the animals and the severity of pathological changes graded.

The purpose of the study was to quantify the histopathologic changes in the lung after inhalation injury, and study the effect of steroid treatment on mortality and lung histopathology. In the placebo group 8 out of 12 died and in the steroid group 5 from 24. This difference was significantly different. But when histological scores were compared, there was no difference between the steroid and placebo treatment groups. Steroids failed to attenuate the pathological changes studied. In fact pulmonary congestion was generally more severe in the steroid treated group. Survival in the steroid treated group was not related to protection of pulmonary ultrastructure.

Liver histology.

There have been few studies of liver histology from patients with ARDS, due to the danger to life of the patient in taking the necessary tissue samples. In animal models of the ARDS like condition, few researcher have focused on liver ultrastructure. We studied hepatic ultrastructure in rabbits (Tighe et al. 1989) and pigs (Tighe et al. 1990) from biopsies taken 2 and 5 hours after the induction of peritonitis. Figure 9 shows a TEM of tissue taken from pig liver before infection was initiated. Figure 10 shows a SEM of similar tissue. The hepatic architecture is clearly defined in both pictures. Figure 9 shows a normal sinusoid lined with endothelium and an apparent space of Disse. Figure 10 shows open sinusoids and clear bile canaliculi.

In both species, after 2 hours there was a 3 fold increase in the number of neutrophils in the hepatic sinusoids without any apparent damage. But after 5 hours the sinusoids appeared occluded with poorly defined architecture as can be seen in the TEM in Figure 11 and the SEM in Figure 12. Figure 11 demonstrates a sinusoid 5 hours after infection, showing dense occlusion by degranulating neutrophils, lymphocytes, cell debris and fibrin deposits. Kupffer and endothelial cells are swollen with consequent narrowing of the sinusoidal lumina and the space of Disse, with a reduction in sinusoidal luminal area. Figure 12 shows occluded sinusoids with poorly defined bile canaliculi.

Figure 13 is an SEM of a normal sinusoid, lined with fenestrated endothelium, through which the space of Disse can be seen. Figure 14 is a SEM of pig hepatic sinusoid 5 hours after infection, it reveals the sieve plates of the sinusoidal endothelium to be disrupted by areas of larger fenestrations. Figure 15 is a similar tissue sample as Figure 14, it shows a swollen Kupffer cell obstructing a bifurcation in the sinusoid. Immediately adjacent to this cell can be seen swollen endothelium, not observed further

away from this cell. This suggests that the swelling is a result of Kupffer cell activation.

Most of the occlusion by leucocytes, Kupffer cells, endothelial cells and fibrin deposition occurred in sinusoids of zone 1 of the Rappaport acinus system (Rappaport 1973). Sinusoids of zone 2 appeared constricted but with little damage whilst the sinusoids of zone 3 appeared more patent where they enter the hepatic vein. Each acinus defines an area of liver tissue organised around the terminal branch of the portal vein in such a way that cells at the centre of the acinus, zone 1, are the first to receive blood, followed by zone 2, and cells located toward the periphery and collecting vein, zone 3, being the last to receive blood (Shaw et al. 1980).

Sinusoidal endothelium is swollen even when not associated with leucocytes. These observations suggest that the inflammatory response is occurring independently of neutrophils. This swelling of the endothelium reduces blood flow through the sinusoids, a feature that together with Kupffer cell swelling may act as a potent control of liver blood flow.

Balis et al. (1978) infused *E. coli* endotoxin into Rhesus monkeys for 22 hours. After 3 hours they noted sequestration of neutrophils into hepatic sinusoids. Over the next 19 hours activated Kupffer cells and neutrophils were seen in the sinusoids. Lymphocytes, monocytes and fibrin were also present in the sinusoids.

Liver function tests are a poor indicator of hepatic dysfunction. The liver can show very severe damage at the histological level without any apparent signs of physiological failure. Animal studies have shown that the lactic acidosis seen in septic shock may be the result of its poor hepatic clearance by the failing liver.

We (Tighe et al. 1989) have shown in pigs that lactic acidosis can be reversed in faecal peritonitis by using drugs that increase hepatic blood flow, without any major increase in oxygen consumption. The lactic acidosis was a function of reduced liver blood flow and is an indicator of its

immanent failure. Daniel et al. (1976) showed that an important feature in developing lactic acidosis in endotoxic shock was the reduction in hepatic clearance of lactate. Similarly, Almenoff et al. (1989) showed that in subjects with hepatic dysfunction serum lactate levels rose faster and remained elevated longer than normal subjects when undergoing exercise tests. Similar results were observed by Connor et al. (1982) when increased blood lactate was attributed to its reduced hepatic clearance.

Spleen histology.

Balis et al. (1978) infused *E. coli* endotoxin into baboons and showed fibrin in the spleen of the monkey. We studied spleen histology in rabbits (Tighe et al. 1989) and pigs (Tighe et al. 1990) in which faecal peritonitis was induced. The spleen shows degranulating neutrophils in the red pulp, arterioles and venous sinuses. Many aggregates of platelets are seen in the red pulp although there was no capillary occlusion. Macrophages showed evidence of activation and contained large amounts of phagocytosed cell debris. Fibrin was also found adjacent to the endothelium of the venous sinuses and red pulp. There was no damage to the endothelium.

Renal Histology.

Coalson et al. (1977) showed that the glomeruli contained multiple fibrin thrombi, disrupted platelets and the glomeruli capillary endothelium was focally oedematous and disrupted in their septic baboons. However, when Balis et al. (1978) infused *E. coli* endotoxin into baboons (1978) they were unable to demonstrate fibrin in the kidney of the monkey. We could not show any histological changes in the kidney of the rabbit or pig with peritonitis.

Heart histology.

Coalson et al. (1977) reported myocardial capillary endothelial oedema and fluid accumulation in interfibre and intrafibre spaces. The studies of Balis et al. (1978) and Tighe et al. (1989) were unable to show leucostasis or fibrin in the heart.

Muscle histology.

No changes to capillary ultra-structure or aggregates of leucocytes have ever been reported in animals or humans with multi-system organ failure.

Conclusion.

Animal studies were performed to produce a more controlled development of the condition. From these studies it was possible to show that the major changes were taking place in the pulmonary capillaries around the alveolar spaces. Leucocytes were aggregating in the capillaries, causing occlusions, whilst the capillary endothelium and alveolar epithelium showed considerable damage.

Researchers such as Coalson et al. in 1977, made the most important discovery, not previously found in ultrastructural studies of patient tissue. They were able to show that, at the same time such changes were taking place in the lung, similar destructive changes were occurring in the liver, spleen and adrenal glands. ARDS was only the pulmonary aspect of multi-system organ failure. These other organs showed similar leucostasis and endothelial disruption.

Chapter 5.

Inflammatory cells.

1. Polymorphonuclear leucocytes.

2. Monocytes.

3. Macrophages

1. Polymorphonuclear leucocytes (neutrophils).

Introduction

Neutrophils are circulating marrow-derived phagocytes, which are short-lived end-stage cells, having a blood half-life of 6-8 hours. According to Weiss and Regiani (1984) they have a significant marginal pool, characterised by moving along the vessel wall, instead of through the central part of the lumen. The result of this characteristic is that when a blood sample is taken to estimate the number of neutrophils, many will not be counted. When a subject is stimulated and the blood flow rate increased, the marginating pool will be decreased, giving the false impression that there is an increase in the number of circulating neutrophils.

They are the first myeloid cell to arrive at an inflammatory focus, where they modulate the process of inflammation, both by amplification and then restriction. In response to specific signals, such as the complement fragments C5a and C3a, leukotriene B₄, or formyl peptides released from bacteria, neutrophils are released from the bone marrow into the circulation, where there is then increased neutrophils activation. The neutrophils aggregate and adhere to endothelium, migrating into the tissue (diapedesis) and then to the site of inflammation (chemotaxis). Once in the tissue, they ingest opsonised micro-organisms, destroying them by the release of lysosomal granular constituents or by active synthesis of toxic oxygen metabolites such as hydrogen peroxide or the hydroxyl radicals.

Dahinden and Fehr (1983) and Wilson (1985) showed that neutrophils represent the most important inflammatory response to infection. But, unfortunately they can produce inflammation amplification, as in septic

shock, that may potentiate host injury. Endotoxin, released in gram negative septicaemia can inhibit chemotaxis, augment adherence to endothelium, promote degranulation and induce synthesis of neutrophil-toxic oxygen species.

Work done by Athens et al. (1961) and Boggs et al. (1964) showed that there is a dynamic interaction between neutrophils and the endothelial wall of blood vessels. About 10% of vascular neutrophils emarginate and move across the endothelium but do not re-enter the circulation in any significant numbers. Wintrobe and Lee (1981) demonstrated that neutrophils were removed via the reticulo-endothelial system of the lung, liver and spleen as well as via the urine and the G.I. tract. This interaction occurs only between the emarginating pool of neutrophils and endothelium. Meyrich and Brigham (1983) showed that neutrophils especially emarginate in the pulmonary circulation of sheep when challenged by endotoxin, possibly because 50% of vessel endothelium is in the lungs. This emarginating pool represents about 50% of the total number of blood neutrophils. These can be mobilised rapidly into the circulating pool by exercise or an infusion of adrenaline. It is unlikely that these emarginating neutrophils are actually adherent to the vessel wall, Mayrovitz et al. (1977) found that when examined by microscopy, neutrophils appear to roll along the endothelial surface.

The aggregation of leucocytes in the lung capillaries (leucostasis) and their disappearance from the peripheral blood (leucocytopaenia), during endotoxic or septic shock were first described by Goldscheider and Jacobs in 1894 and Webb in 1924. Since this early work many studies have shown these phenomena in animal studies, Wittels et al. in 1974, Powe et al. in 1982 and Snapper et al. in 1983, resulting in leucocytopaenia and pulmonary sequestration in several species. This combination of organ sequestration and degranulation by neutrophils appears to be a major cause of inappropriate tissue damage in response to septic shock.

In our laboratory, we (Tighe et al. 1989) have shown similar results, using a rabbit faecal peritonitis model of ARDS. Figure 16 shows the blood count of neutrophils in two groups of animals, each containing 6 rabbits. One group was sham operated and did not receive a faecal peritonitis infection, the other received such an infection. Blood samples were taken at baseline in both groups. They were then taken hourly for five hours. A precipitous fall to 12% of baseline, in the number of circulating neutrophils can be seen in the infected group, whereas there was a 30% increase in the sham operated group. Lung tissue was taken from both groups at baseline and after five hours, and prepared for electron microscopy. The number of neutrophils were counted per alveolae, in each group of rabbits studied. Figure 17 shows there was no change in the number of pulmonary neutrophils in the sham operated group, but there was a five fold increase in the infected group. We (Tighe et al. 1989) have also shown, described in the next chapter, that neutrophils are sequestered in the liver and spleen, but not kidneys, heart or muscle.

Figure 16. Peripheral blood neutrophil count in 6 sham operated and 6 septic rabbits over a five hour period. (Tighe et al. 1989)

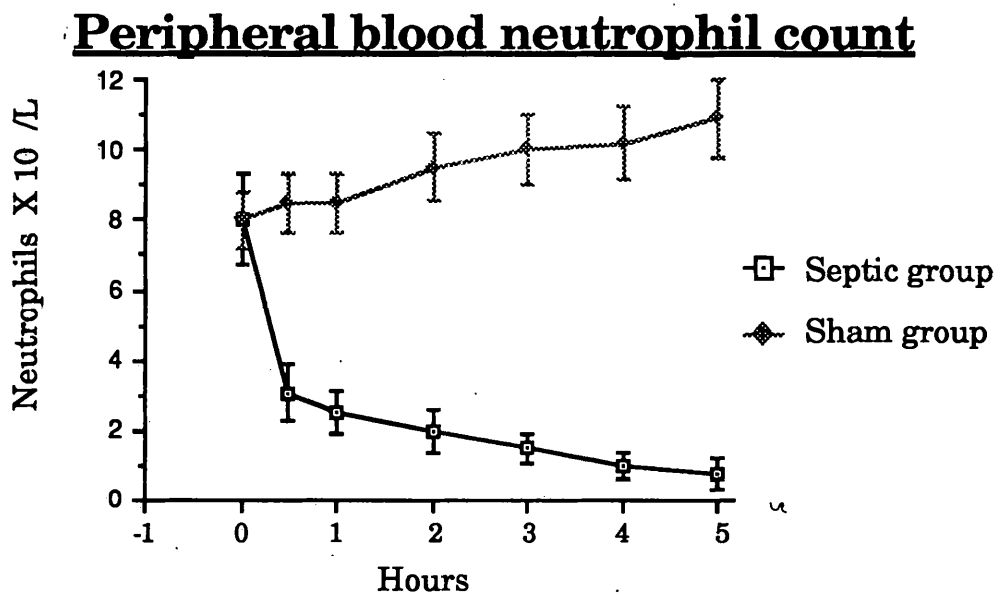
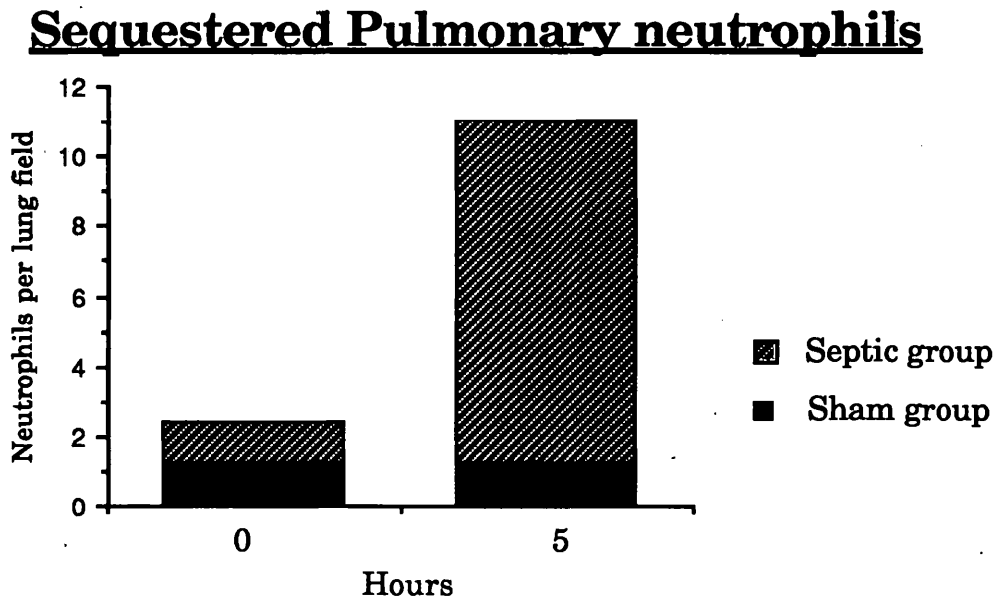


Figure 17. Number of neutrophils per alveolae in sham and septic rabbit lung at baseline and after five hours of peritonitis. (Tighe et al. 1989)



Wittels et al. (1974) challenged monkeys with *E. coli* endotoxin, and found that the peripheral leukocyte count fell by more than 50% in 30 seconds. Lung sections taken one hour after endotoxin infusion, showed large numbers of leucocytes in the pulmonary capillaries. At the end of the study considerable pulmonary damage was observed. Begley et al. (1984) found a fall in circulating neutrophils in sheep, from a control value of $8,000 \times 10^9/L$ to $2,000 \times 10^9/L$, after 30 minutes of endotoxin infusion. He also found that neutrophils in the pulmonary alveolae rose from a control value of 1 to 3.75 after 30 minutes, and to 6 after 4 hours.

The number of neutrophils in bronchial lavage has been shown to be predictive of abnormalities in gas exchange and lung protein permeability. Weiland et al. in 1986 demonstrated that, in ARDS patients, neutrophils constituted 67% of recovered lavage cells, whereas in control mechanically ventilated patients they were only 4%, and in normal volunteers 0.8%. These data suggest that the neutrophils seen in the pulmonary capillaries migrate through to the alveolar sac.

Although it might be attractive to assume that the migrating neutrophils cause pulmonary damage and increase capillary permeability, it is unlikely. Movement of neutrophils through a capillary wall do not usually cause increased permeability. Work performed by Renaldo and Rogers 1982 dissociated the change in permeability caused by endotoxemia, from the migration of neutrophils out of the pulmonary capillaries into the air spaces. They injected endotoxin into the peritoneum of rats, finding the usual immediate increase in capillary sequestration of neutrophils and an increase in the retention of I^{125} albumen in the lungs after 6 hours. But, by 24 hours the iodinated albumen retention levels in the lung had returned to normal. Bronchial lavage showed only a small increase in neutrophils at 15 hours, and at 24 hours there was a marked increase in their number. The recovery of neutrophils remained significantly elevated for 3 days and only returned to normal values at day 6.

Two phase response of pulmonary dynamics.

Snapper et al. (1983) studied the effect of an infusion of *E. coli* endotoxin in sheep. They attenuated its effects, by infusing the prostaglandin inhibitors, meclofenamate or ibuprofen. They were able to show that pulmonary changes occurred in two phases. The first phase was characterised by pulmonary hypertension and could be inhibited by the action of meclofenamate or ibuprofen. From these data, they inferred that it was mediated by thromboxane, causing vascular constriction. The second phase showed an increase in lymph flow and was associated with leucostasis, but could not be inhibited by these drugs. The initial phase of pulmonary hypertension is separate from the pulmonary leucostasis that occurs later. Begley et al. (1984) in a sheep model of ARDS similar to that of Snapper et al. (1983) studied the effects of steroid and non-steroidal anti-inflammatory drugs on the pulmonary responses to *E. coli* endotoxin infusion. They found that steroid pre-treatment could prevent the increased

pulmonary capillary permeability associated with leucostasis, by preventing the pulmonary leucostasis from occurring.

Leukocytopaenia.

Although leucocytes may be important in organ damage, the work described above does not confirm whether these changes were causes or effects. Further work was performed on neutropaenic animals to establish the importance of neutrophils in pulmonary dysfunction. Pingleton et al. (1975) compared the pulmonary ultrastructure of endotoxic shocked rhesus monkeys made neutropaenic by whole body irradiation, with similarly treated non-irradiated animals. Electron microscopy demonstrated pulmonary capillary sequestered neutrophils in the non-irradiated group, but a complete absence in the irradiated group. Both groups had significant endothelial swelling and perivascular oedema. Leukocytopaenia provided no protection from the histological damage induced by endotoxin. Gaynor (1973) also showed that damage to the pulmonary vessel walls arose after the administration of endotoxin, even in animals made leukocytopaenic by nitrogen mustard treatment.

Brigham et al. (1979) and Heflin and Brigham (1981), who measured lung lymph flow as an index of pulmonary damage in sheep, found that leukocytopaenia significantly reduced lymph flow. But neither performed any histological studies in their sheep and therefore do not know what changes occurred in the endothelium. Both of these groups, using dogs or sheep, found that the increase in pulmonary vascular resistance was independent of the presence of neutrophils. This supports the contention of Snapper et al. (1983) regarding the two phase response of pulmonary hypertension mediated by prostaglandins and increased lymph flow mediated by neutrophils. But this contention obscures the involvement of neutrophils in causing endothelial disruption and pulmonary degradation. None of the studies completely remove all of the neutrophils and none of

them continued for sufficient length of time to establish the degree of damage that could occur in the presence of very small numbers of neutrophils. There could be a third phase, in the pulmonary response to *E. coli* endotoxin infusion, involving endothelial swelling and perivascular oedema, that is independent of neutrophil action. This characteristic could be more widespread than the lungs and liver, where neutrophils aggregate. It could mean that endothelial damage is occurring in other organs, such as muscle, heart and kidney.

Patients with ARDS have characteristics similar to experimental animals with neutropaenia, that is, the disappearance of neutrophils from the peripheral circulation and pulmonary leucostasis, the static accumulation of neutrophils in the pulmonary capillaries. But, there is a group of patients who develop ARDS, with the commonly accepted clinical and X-ray criteria for this syndrome, who are also neutropaenic. Patients receiving suppressive or cytotoxic drugs, such as patients with leukaemia, become neutropaenic, but many also develop ARDS. Neutropaenia provides no protection against developing the condition. Maunder et al. (1986) described 4 neutropaenic patients who developed ARDS and showed typical histological changes associated with the condition. Rinaldo and Borovetz (1985) described 6 neutropaenic patients who developed ARDS. In four patients, there was a worsening of the condition with a fall in arterial oxygenation, when their neutrophil count returned to normal values.

Neutrophil role in endotoxin induced endothelial cell injury.

Endotoxin induced endothelial cell injury has been implicated in the pathogenesis of different diseases such as septicaemia. But whether endotoxin can cause injury directly to endothelial cells or whether mediators are necessary for the injury to occur is uncertain. Harlan et al. (1983) studied bovine pulmonary endothelial monolayers using electron microscopy. They brought them into contact, *in vitro*, with endotoxins and

showed dilatation in their intracellular junction and cellular changes representing contractions. Two hours after incubation with endotoxin cell death was found. These findings show that endotoxin mediates directly or complements independent endothelial cell cytotoxicity and this injury is not prevented by inhibitors of protein and prostaglandin synthesis. However, according to Meyrick et al. (1986) and Yamada et al. (1981) this endothelial injury observed after incubation of endotoxin with endothelial cells in culture can only be observed with bovine endothelial cells but not with human cells. Harlan et al. showed (1983) that if human endothelial cells in culture are incubated with endotoxin in the presence of granulocytes, these cells adhere to and significantly injure cultured endothelial cells. This injury can be reduced by suppression of granulocyte adhesiveness or by free radical scavengers. Human endothelial cells were seen by Morel et al. (1986) to become susceptible to endotoxin-induced cell toxicity when low density lipoproteins (LDL) are present, forming a complex with LDL, endotoxin can enter endothelial cells and kill them.

Workers have shown that although endotoxin can damage bovine endothelium, it does not directly injure human endothelium. Injury to human endothelium, in response to endotoxin, requires the presence of an intermediate such as neutrophils or LPL.

Pulmonary leucostasis without damage.

Pulmonary leucostasis has been shown by Webster et al. (1982) to occur without functional lung changes. They administered cobra venom factor, zymosan or C5a to rabbits without surgery, anaesthesia or any other intervention. They discovered that although all these factors cause peripheral leucocytopaenia and pulmonary leucostasis, no lung inflammation occurred, as measured by vascular permeability, neutrophil diapedesis into the alveolae or increased pulmonary vascular resistance. After 24 hours, the neutrophils started to move out of the lungs. When

combined with traumatic interventions, such as surgery or anaesthesia, pulmonary damage did occur. Pulmonary leucostasis alone does not inevitably produce pulmonary damage. Work performed *in-vitro*, by O'Flaherty et al. (1979) showed that although arachidonic acid can stimulate neutrophils to aggregate, it does not cause any lysosomal degranulation, as measured by lactic acid dehydrogenase and lysozyme. Clearly there are two phases to the damage that results from the action of neutrophils. First they congregate in the organ's capillaries in close proximity to the endothelium, then they become activated to damage the endothelium and move into the tissue. It is possible that the neutrophil has two separate receptors, one for aggregation and one for degranulation.

Leukocyte adhesion.

When Tonneson et al. (1984) reduced neutrophil adherence to cultured endothelium either pharmacologically or mechanically, cell injury by activated neutrophils was reduced. When Boogaerts et al. in 1982, increased neutrophil adherence to cultured endothelium, endothelial cell cytotoxicity was increased.

Close proximity between the neutrophil and endothelial cell appears to be required for cytotoxicity, reflecting the need for a micro-environment at the cell-cell interface, which is protected from exogenous scavengers. This neutrophil/endothelial interaction is radically altered when acute inflammation occurs, as shown by Grant (1973). This adherence is therefore a prerequisite for extravascular migration to the site of inflammation. But it may also be of greater importance in the pathogenesis of vascular and tissue injury with acute inflammation.

Influence of leucostasis

The mechanism of leucostasis was initially investigated by looking at the adhesiveness of neutrophils in patients with ARDS and sepsis.

Zimmerman et al. (1984) assayed granulocyte adherence to nylon fibres using whole blood collected from the pulmonary artery of 14 patients within 24 hours of satisfying the criteria for ARDS. The mean value of adherence was 83% of that of normal subjects. In 10 of the 14 patients it was equal to or less than controls. A similar method was used by Venezio et al. (1982) who looked at bacteraemic patients with and without shock and compared them to normal controls. The neutrophil adhesiveness of bacteraemic non-shocked patients was similar to controls, but in bacteraemic shocked patients it was raised by 20%. Enhanced adhesiveness was demonstrated when normal neutrophils were suspended in plasma from shocked hypotensive patients. Methylprednisolone significantly reduced the adherence of neutrophils. These data were collected at an earlier time in the course of the syndrome than those of Zimmerman et al (1984). The bacteraemic patients had not yet developed ARDS, and pretreatment with steroids is known to prevent pulmonary damage associated with endotoxin. It is possible that there is an early short term increase in neutrophil adhesiveness. But our work using two separate techniques to measure adhesiveness in rabbits and pigs showed no significant changes in adhesiveness (Tighe et al. 1990, 1989).

Tighe et al (1989) and Baylis et al. (1978) showed that neutrophils aggregate in the sinusoids of the liver and spleen. Tighe et al (1989) and Baylis et al. (1978) further showed that neutrophils do not sequester in the heart, kidney or muscle. If leucostasis was due merely to an increased adhesiveness, then neutrophils would be expected to adhere to all endothelium in all organs. That neutrophils selectively aggregate in the lungs, liver and spleen suggests that there is a neutrophil and endothelial interaction.

These three organs are also part of the reticulo-endothelial system (RES), raising the possibility that damaged or eccentric neutrophils are being

cleared from the circulation, but their numbers being so great, overwhelm the RES and cause organ damage.

Endothelial-leukocyte adhesion molecule (ELAM) and leukocyte adhesion inhibitor (LAI).

Bevilacqua et al. in 1987 discovered that monokines play an active role in neutrophil endothelial adhesion. They reported that purified natural human monocyte derived interleukin 1 can act directly on cultured human endothelial cells (HEC) to increase dramatically the adhesiveness of their surfaces for human neutrophils, monocytes and their cell lines HL-60 and U937. This effect was shown to require protein/RNA-synthesis and be mediated primarily through the endothelial cell. They developed a series of murine monoclonal antibodies directed against monokine-stimulated HEC surfaces. One of these antibodies (H4/18) recognises an endothelial cell surface structure which is induced by IL-1 and certain other cytokines in a similar fashion to the pro-adhesive surface changes for leucocytes. H4/18 partially blocks HL-60 cell adhesion to monokine treated HEC, and, *in vivo*, labels human vascular endothelial sites of delayed hypersensitivity reactions. A second monoclonal antibody (H18/7) significantly blocked the adhesion of both HL-60 cells and neutrophils to monokine treated HEC. Thus H4/18 and H18/7 appear to recognise the cell surface structures on HEC responsible for adhesion. They designated this surface structure, "endothelial-leukocyte adhesion molecule" (E-LAM-1). IL-1 treated HEC cultures generate a leukocyte adhesion inhibitor (LAI). LAI acts on neutrophils to inhibit their adhesion to hyperadhesive endothelial monolayers, but does not inhibit neutrophil activation by chemotactic stimuli. LAI appears to inhibit adhesion of peripheral neutrophils and monocytes, but not lymphocytes, to hyperadhesive HEC. This endothelial derived inhibitory activity blocks leucocyte adhesion without suppressing leukocyte function.

The work of Gimbrone et al. (1987) showed that the endothelium is the source of neutrophil adhesion, generating proteins that either enable adhesion to take place or inhibit their adhesion. What this work does not address is why pulmonary and hepatic endothelium should allow leucocytes to adhere in cases of septicaemia and multiple trauma. It is possible that the endothelium of differing organs have variable enzyme systems producing different levels of E-LAM and LAI.

Neutrophil mediated tissue injury

Three classes of mediators are likely to be involved in the pathogenesis of neutrophil/endothelial damage: granular enzymes, reactive oxygen metabolites and membrane phospholipases.

1. Granular enzymes.

Granular enzymes are produced in the azurophilic and secondary granules. Lysosomes contain a number of substances capable of mediating vascular injury. Cationic lysosomal proteins increase vascular permeability *in vivo* as shown by Janoff et al. in 1965 and 1968 and Cockrane and Aiken in 1966.

Elastase is a major granular enzyme secreted by neutrophils capable of dissolving elastin, reported by Harlan et al. in 1981 to detach cultured endothelium from culture substrate *in vitro*. Endothelial membrane components or the subendothelial matrix can be degraded by elastase in the presence of plasma protease inhibitors due to the protected micro-environment at the interface between the neutrophil and the endothelium. Endogenous plasma alpha-1-protease, the elastase inhibitor, was shown by Weiss and Regiani in 1984 to be inactivated by neutrophil derived oxidants, allowing damage to occur. Bronchial lavage from patients with ARDS has been found by Lee et al. in 1981 to contain free neutrophil elastase, suggesting it may be involved in the pathogenesis of vascular injury *in vivo*.

Fowler et al. in 1984, compared lysosomal enzyme release of neutrophils isolated from control and ARDS patients. The neutrophils from ARDS patients showed a fourfold increase. He further showed that normal neutrophils exposed to the plasma of ARDS patients did not show raised activation or enzyme secretion level, suggesting that the activating agent was not blood-borne but of cellular origin.

2a. Reactive oxygen metabolites.

Oxidants or toxic oxygen metabolites according to Sachs et al. in 1978 could be mediators of cellular injury. These were shown to include superoxide anion, hydrogen peroxide and hydroxyl ion by Wiess and LoBulgio in 1982. These oxygen metabolites can initiate membrane peroxidation and damage intracellular components. Work performed *in vitro* by Wiess et al. (1981) and *in vivo* by Till et al. (1982) showed that toxic oxygen metabolites derived from neutrophils, particularly hydrogen peroxide or its products, induce endothelial cell lysis. Zimmerman et al. (1984) looked at the production of superoxides, *ex vivo* in neutrophils from eight patients suffering from ARDS. Four patients had raised super oxide levels, and four had normal or reduced levels.

The work of Hinshaw et al (1989) has shown that oxidants are unstable oxygen metabolites such as O_2^- , H_2O_2 , and OH^- , having potent oxidising properties which can alter cell function. Most oxidant induced direct cell injury appears to be caused by OH^- . The OH^- is in large part produced by conversion from O_2^- and H_2O_2 which occurs in the presence of free iron (Fe^{3+}). Tissue damage can be measured at several levels. At the subcellular level, DNA alteration is seen. At the cellular level, a peroxidation of the lipid moiety of the cell membrane occurs, which alters membrane and membrane enzyme function. Increased vascular permeability can also result, although this process may be as much related to the oxidant-decreasing antiprotease defenses rather than direct OH^-

attack on the microvascular membrane. Oxidants, in particular OH^- , also activate complement with additional activation of other cascades, in particular the arachidonic acid cascade. Lipid hydroperoxides generated by oxidants are extremely potent activators of the cyclo-oxygenase enzymes.

2b. Other sources of oxidants.

Although neutrophils and macrophages are an important source of oxidants through intracellular myeloperoxidase activity, it must be remembered that the endothelium also produces oxidants in response to reperfusion after tissue ischemia, by the action of xanthine oxidase (McCord 1985). The concentration of xanthine oxidase in tissue is usually low, but it is converted from xanthine dehydrogenase, during cell ischemia. On reperfusion, xanthine oxidase acts on the large cellular content of hypoxanthine in the presence of oxygen to produce O_2^- and H_2O_2 , which in turn can result in further oxidant reactions. This factor produces a major clinical problem. If blood flow to major organs is decreased during shock, causing damage through ischemia, then when blood flow is restored to an organ the potential damage to the organ is even greater. It should also be emphasised that this mechanism of superoxide production, could cause tissue damage in the absence of neutrophils, and could cause injury in those organs that do not show leukocyte aggregation.

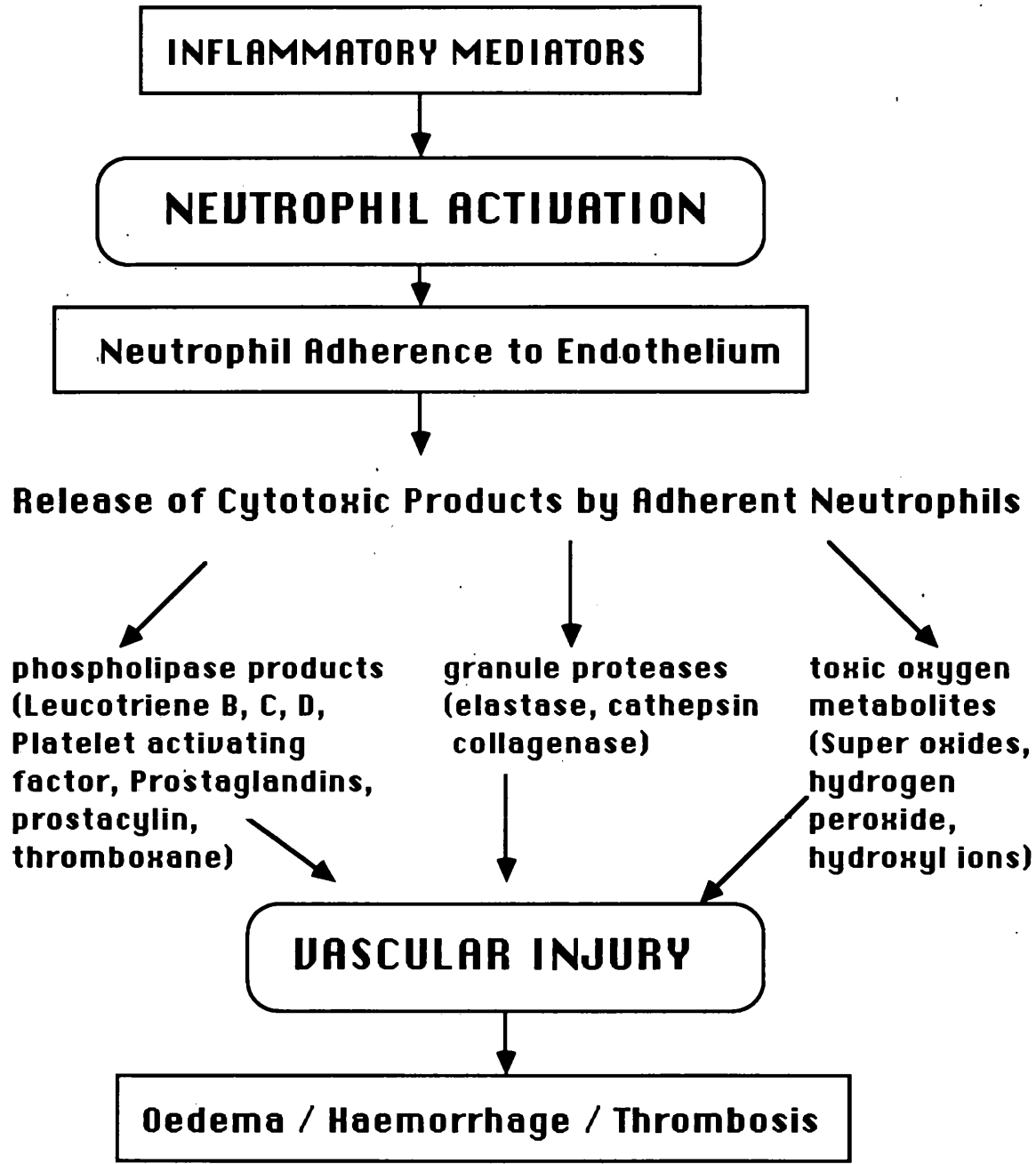
3. Membrane phospholipases.

Harlan (1984) showed that phospholipase A_2 provokes endothelium to release the arachidonic acid metabolites prostacyclin, prostaglandin E_2 , thromboxane, leukotrienes B, C and D. and platelet activating factor. The considerable importance of these inflammatory mediators will be discussed in a later chapter.

There is little doubt that neutrophils have the ability to cause considerable tissue destruction and either release or induce the release of

numerous active inflammatory agents, by the three mechanisms mentioned above.

Figure 18: Summary of possible mechanism of vascular injury caused by neutrophil activation.



Monocytes

Monocytes are circulating marrow-derived phagocytes, with a blood half-life of 71 hours and apparently do not have a significant marginal pool according to Weiss and Regiani (1984). Most importantly blood monocytes are destined to become long-lived tissue macrophages.

Like neutrophils, monocytes must first adhere to endothelium before migrating from the blood stream to extravascular sites. Complement activation *in vivo* induces a transient monocytopenia as shown by McGuire and Spragg in 1982. Sacks et al. showed in 1978 that isolated monocytes *in vitro* aggregate to chemotactic factors. It is likely that the monocytes behave like the neutrophils *in vivo* by undergoing aggregation and margination. The process by which adherent monocytes diapedese across intact endothelium *in vivo* is similar to that observed in neutrophils according to Huttemeier et al. (1982). Weiss and Regiani (1984), showed that monocytes accumulate in skin windows more slowly than neutrophils, but are the predominant cell after 12-24 hours. Monocytes, like neutrophils, secrete factors that are potentially cytotoxic or which may alter endothelial function. There are no reports of mononuclear phagocyte-mediated endothelial injury. Based on studies of neutrophil-mediated endothelial injury, it is likely that mononuclear phagocyte-derived toxic oxygen radicals or proteases will also induce endothelial injury. Like neutrophils, monocytes, also produce a number of lipoxygenases and cyclo-oxygenases capable of altering vascular permeability and vasomotor tone.

In addition to toxic oxygen radicals, proteases, lipoxygenases and cyclo-oxygenases, mononuclear phagocytes release several monokines that affect the vessel wall: tumour necrosis factor and interleukin-1. Tumour necrosis factor, interleukin-1 and alpha interferon production is now considered to be among the most important functions of monocytes in ARDS and MSOF.

Macrophages

Macrophages are derived from the circulating pool of monocytes and are part of the reticulo-endothelial system distributed throughout the body. They are present in the interstitium and alveolae of the lung, peritoneum, liver (Kupffer cells), spleen, bone marrow and lymph nodes. The monocyte moves into the tissue and further differentiates into tissue macrophages. These cells remove and destroy bacteria, damaged tissue cells, neoplastic cells and macromolecules such as antigen-antibody complexes. But macrophages are also more than a non-specific phagocyte. They also inhibit bacterial function, facilitate viral destruction, produce chemotactic factors and most importantly produce tumour necrosis factor and interleukin-1.

Conclusion.

Neutrophils are certainly important in the development of ARDS and MSOF. They have the destructive ability to cause considerable tissue damage. In animal models, of ARDS, there is a precipitous fall in the number of peripheral neutrophils when challenged by endotoxin or infection. They are sequestered in the lungs, liver and spleen but not kidney, heart or muscle. There appears to be an interaction between the endothelium of certain organs and neutrophils that causes leucostasis. Pulmonary leucostasis can occur without lung damage.

The oedema formation seen in animal models of ARDS although associated with leucostasis is not the cause. Oedema formation occurs in neutropaenic animals and its time course does not coincide with leucostasis.

Although neutropaenia attenuates pulmonary damage in animal models of ARDS, neutropaenic patients develop ARDS which is often lethal. It is possible that these patients are never totally neutropaenic and have a small number of neutrophils in the circulation. This possibility seems unlikely because there would be no quantitative association between the

number of neutrophils and the degree of tissue damage, i.e. a very small number causes the same injury as large numbers.

A major problem in looking at blood borne inflammatory cells, is to consider that the behaviour of cells in the blood during ARDS is the same as those cells causing damage in the tissue. There could be two populations of neutrophils or monocytes: one group of cells causing damage in the tissue, while the other is inactive and does not cause such damage.

This evidence suggests that although neutrophils are important in the inflammatory process, they are not the principle cause of whole body inflammation and the development of ARDS and MSOF, although they undoubtedly accentuate tissue damage. The importance of macrophages is in their production of tumour necrosis factor discussed in detail in chapter 6, section 1b.

Chapter 6.

Biochemistry of inflammation.

Section 1. Protein mediators.

1a. Kinins.

1b. Cytokines

1c. Complement.

Section 2. Lipid mediators.

2a. Phospholipids

2b. Phospholipase A2.

2c. Arachidonic acid.

2d. Platelet activating factor.

2e. Cyclo-oxygenases

2f. Thromboxane/Prostacyclin.

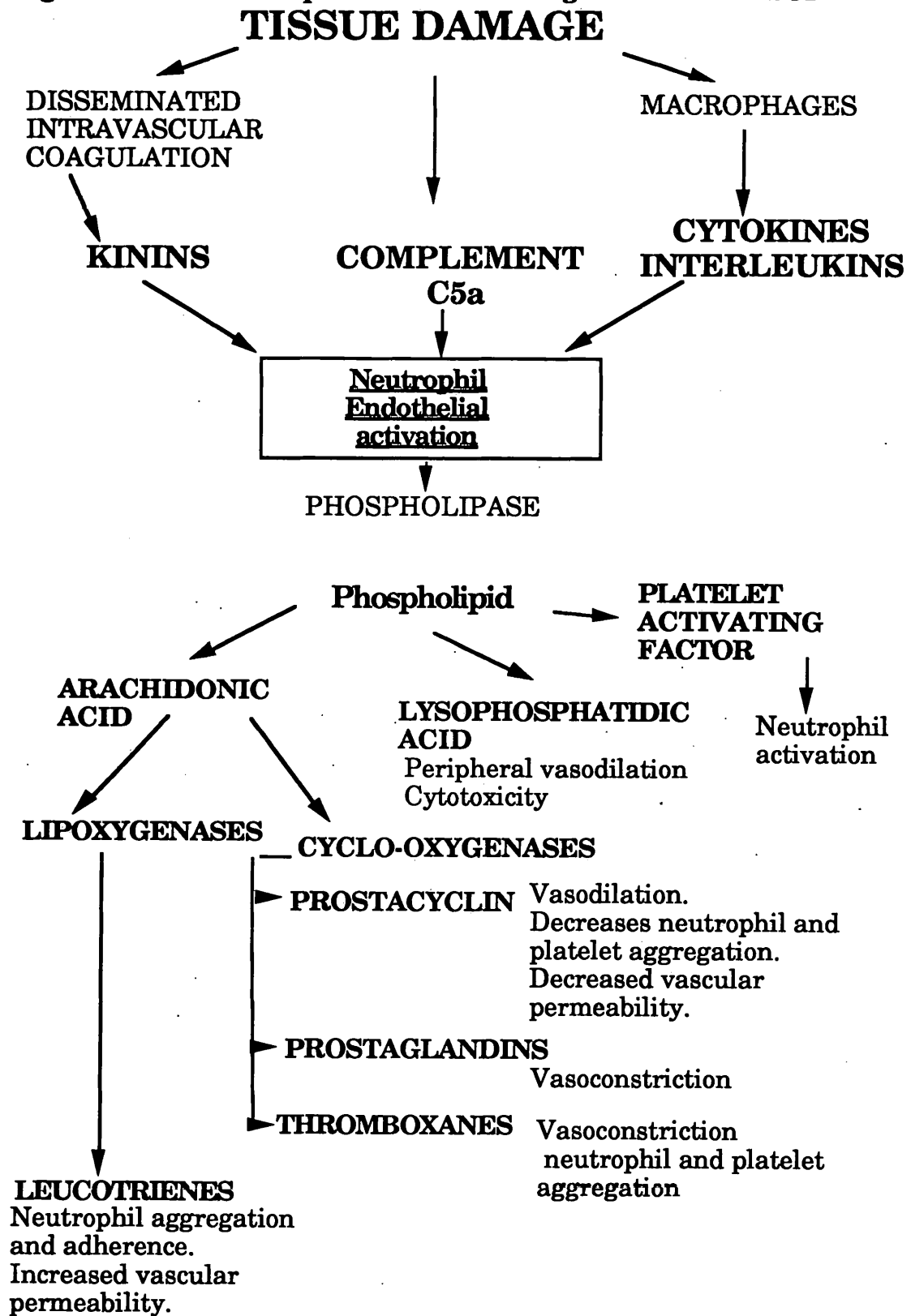
2g. Lipoxygenases

Introduction

The inflammatory cascade shown in Figure 19 is initiated by a wide variety of stimuli. They include tissue damage, hypoxemia and ischemia, endotoxin, and exogenous pathogens such as bacteria, virus and fungi. The first part of the process involves the protein elements of plasma, such as kinins and complement. In addition, tumour necrosis factor and interleukin-1 are released from stimulated inflammatory cells, such as neutrophils, monocytes and tissue macrophages. Although other mediators such as histamine and 5-HT are undoubtedly released, they are non-specific in the development of ARDS and MSOF.

When the inactive protein elements of the kinin and complement systems in the plasma contact the inflammatory stimuli they become activated. The kinins and complement stimulate both the endothelium and a further number of circulating inflammatory cells. The circulating inflammatory cells have two sources of activation.

Figure 19. Mediator response to tissue damage involved in MSOF



When monocytes, tissue macrophages and neutrophils (Yoshinaga et al. 1987) are stimulated, they also produce the cytokines; TNF and the interleukins. These cytokines further activate the endothelium. This system of activation can result in a whole body inflammatory reaction to the initial insult, by blood-borne mediators.

The endothelium and circulating inflammatory cells are stimulated through the cell membrane constituent, phospholipase, to initiate the lipid inflammatory cascade. These lipid mediators, unlike complement and kinins, are thought to act locally. They include platelet activating factor, thromboxane, prostacyclin and the leukotrienes. These inflammatory mediators actually cause tissue damage, this tissue damage causes further mediator activation and further damage. This system of inflammatory amplification causes organ disfunction to occur. Even when the initial insult is removed or blocked, tissue damage caused by the inflammatory response can maintain and further amplify the organ damage. If the initial insult is quickly removed then the cascade can be stopped, but if allowed to continue, fresh tissue damage will be caused, further stimulating the system to whole body inflammation. Once this level of activity has been reached it is unlikely to be stopped by any of the body's natural systems.

Section 1.

Protein mediators of inflammation.

1a. Kinins.

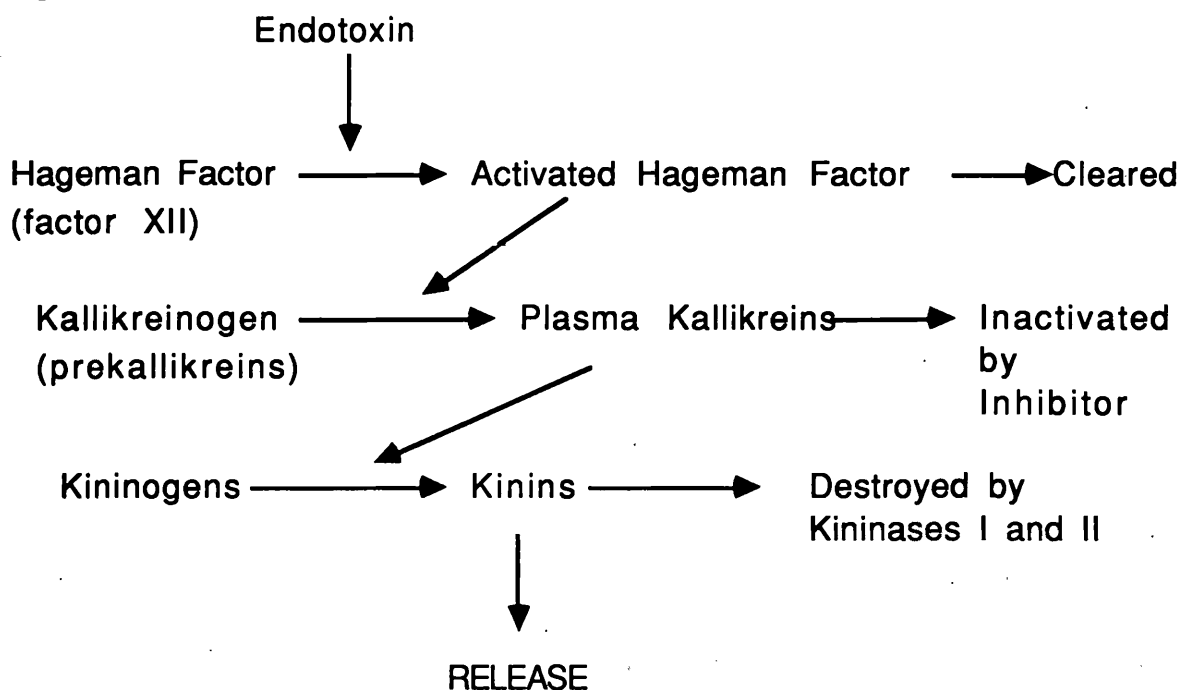
Introduction

Bradykinin Blockade.

Wilson et al. in 1989, tested the hypothesis that bradykinin (BK) plays an important role in the pathology of endotoxic shock by studying the effect of a BK antagonist NPC 567 in rats treated with endotoxin. Lipopolysaccharide (LPS) administration resulted in an increase of plasma BK from < 23 pg/ml to 144 pg/ml at one hour. The mean arterial blood pressure fell 38%, while the breakdown product of prostacyclin, 6-keto-prostaglandin-F1-alpha (6kPGF1a) rose from 289 pg/ml to 7,927 pg/ml. NPC 567 partially reversed the fall in MABP and reduced the rise in 6kPGF1a by 42%, suggesting a reduction in prostacyclin production. Most importantly it reduced the mortality from 100% to 50%. These results strongly suggest that BK plays a significant role in the pathology of shock.

Background

Kinins are a family of small peptides formed in blood and biological fluids of mammals by the actions of kallikreins on large protein substrates, the kininogens. In general, the concentration of kinins in circulating blood and tissue is fairly low, since kallikreins are coupled to inhibitors and kept as inactive precursors, prekallikreins. When an appropriate physiological or pathological stimulus activates the prekallikreins, plasma kallikreins are formed. Bradykinin is formed in the blood by the action of plasma kallikreins on the kininogens. Kallidin (KD) is released in tissue by the action of tissue kallikreins on kininogens (Roche and Silver 1980). Kinins are inactivated by kininase II which is particularly abundant in the lung and kidney, and appears to be localised in the plasma membrane of endothelial cells (Erdos 1979, Palmiere et al. 1986).

Figure 20. Scheme of Kinin generation

The metabolites desaturated Arginine (des arg) BK and des Arg KD are generated by the enzyme kininase 1, found in endothelium. Inactivation of kinins is quite efficient at the site of release. The kinins which escape into the circulation are broken down to inactive fragments in the lung, BK by 80-95%, KD by 70%, while des Arg BK, des Arg KD are resistant to degradation (Ferreira and Vane 1967).

Kinins are also inactivated in the kidney and other organs, consequentially their concentration in arterial blood is less than 30pg/ml. (Carretero et al. 1981).

Biological actions of bradykinin and related kinins.

Kinins are naturally occurring peptides, exerting a number of biological actions *in vivo* and *in vitro*. Intravenous BK induce an acute fall in blood pressure which is dose dependant, and rapidly reversible (Regoli 1984). The hypotension is partly due to peripheral vasodilatation, which is brought about by relaxant effects (endothelium dependent) of BK on resistance vessels. The capillary endothelium and the veins are contracted

by the kinins, similar to other smooth muscles from the uterus, the trachea and bronchi, the stomach and the intestine, the bladder and the urethra. (Regoli D. 1984)

Several biological effects of BK are mediated by endogenous agents such as prostaglandins, histamine, 5 hydroxytryptamine, catecholamine and renin (Regoli D. 1980) The release of prostaglandins, can cause an increase in vascular permeability, and hypotension through their vasodilation properties. Among the most significant biological actions of kinins is the activation of neural circuits by the stimulation of sensory nerve endings in various tissues. In dogs and rabbits, BK induces rapid changes in blood pressure through the stimulation of sensory afferents in the rabbit ear or dog splanchnic or carotid arteries (Ferreira et al. 1973). Applied to the portal vein of rats, BK induces the release of vasopressin (Stoppini L, Barja F, Mathison R, Baertchi J. Spinal substance P transmits bradykinin but not osmotic stimuli from hepatic portal vein to hypothalamus in rat. Neuroscience 1984; 11: 903-912) These biological actions of the kinins are due to the activation of at least two different receptor types.

Bradykinin receptor sites

The biological actions of kinins are due to the activation of at least two different receptor types. The B1 receptor mediates the contractile effect of kinins in the rabbit aorta and mesenteric vein, and shows high sensitivity to des Arg BK and des Arg KD. The B2 receptor mediates both the relaxation of the dog carotid artery and the contraction of the rabbit jugular vein, with a high sensitivity to BK and KD.

In vivo, B1 receptors are activated in rabbits by the intravenous injection of *E. coli* endotoxin. In rabbits treated with this endotoxin, des Arg BK (inactive in normal animals) becomes a potent vasodilator and hypotensive agent.

Most of the pathological actions of kinins, such as peripheral vasodilation, have been attributed to activation of the B2 receptors in the endothelium of large and small vessels. The major physiological events of the inflammatory reaction, vasodilation, increase in vascular permeability, venoconstriction and mobilisation of blood and tissue cells, are generally activated more efficiently by BK than by des Arg BK and are not modified by B1 receptor antagonists.

Mechanisms of action of kinins.

Kinins are one of the most potent activators of prostaglandin release (Marceau et al. 1983); for instance endothelium mediated vasodilatation, the smooth muscle contraction or relaxation in various organs, are associated with the release of prostaglandins. Kinins promote the release of prostacyclin from blood vessels of the heart and kidney, from cell cultures (such as rat adipocytes and human endothelial cells), possibly by interacting with membrane phospholipases. This stimulation of prostacyclin production, especially thromboxane and prostacyclin can have a profound effect in the development of MSOF, as will be discussed later in this section.

Involvement of kinins in inflammation.

Acute inflammation was induced in rats by intraperitoneal injection of endotoxin by Kageyama et al. in 1985. The livers of injured rats released twice as much kininogen into the perfusing physiological medium as the livers of normal animals. Once released into peripheral tissue, kinins act on blood vessels, particularly the endothelium through which they produce both peripheral vasodilatation by promoting the release of a smooth muscle relaxing factor and increase of capillary permeability by contracting the capillary endothelium. Kinins may increase intracapillary and venous pressure by contracting the venous smooth muscle. They are therefore

blood-borne pro-inflammatory agents exerting potent actions on blood vessels.

Kinins find specific receptors on leucocytes, and inhibit the neutrophil chemotactic response. Once they enter the inflamed area, leucocytes may perpetuate the production of kinins since they have been shown to contain kallikreins and kinins (Zachariae et al. 1986).

The mechanisms by which kinins exert all these actions are unknown. It is possible that the activation of phospholipase could be involved in some but not all pro-inflammatory effects of these peptides. Over 90% of the biological activity of bradykinin is lost on a single passage through the pulmonary circulation (Ryan et al. 1968). The inactivation is due to metabolism of BK, notably by angiotensin 1 converting enzyme (kininase II) which is localised in the pulmonary endothelium (Ryan et al. 1975, Seager et al. 1983). Angiotensin converting enzyme is responsible for terminating the biological actions of BK and for converting angiotensin 1 into angiotensin 2. These two actions; inactivation of a substance that tends to lower blood pressure and the formation of a potent hypertensive hormone released directly into the systemic circulation from the endothelial cells of the pulmonary vascular bed, play a prominent role in the control of blood pressure (Cochley et al. 1978).

The work of Cochley et al. (1978) shows that BK exerts an hormonal effect on specific receptors possessed by vascular endothelial cells to release 20 fold levels of thromboxane or prostacyclin from bovine pulmonary artery endothelial cells. No platelets are necessary for this action.

Normally plasma contains bradykinin in the order of 1 ng or less per ml, but potentially several thousand times more may be released from the precursor protein kininogen. But as BK is rapidly removed from the circulation, it is very difficult to measure its plasma concentration. The turnover of bradykinin may therefore, be more important. Measurement of kininogen in septic patients, was undertaken by Hirsch et al. in 1974. They

showed that the levels of kininogen fell to low values in those patients who died of septic shock. In contrast, the kininogen levels rose toward normal in those patients who survived. It was thought that the decrease in kininogen was associated with the liberation of kinins by activated plasma kallikrein and the exhaustion of available substrate.

Martinez-Brotons et al. (1987) compared plasma kinin in patients suffering septic shock with those having cardiogenic shock. Patients with septic shock, especially in fatal cases, showed a highly significant decrease in levels of factor XII, prekallikrein, high molecular weight kininogen, alpha macroglobulin and antithrombin. Components and inhibitors of the kallikrein-kinin system were within normal limits in patients with cardiogenic shock. These findings further demonstrate the importance of this system in mediating some of the changes seen in septic shock, and in demonstrating the difference between septic and cardiogenic shock.

Conclusion.

The work on the involvement of bradykinins in ARDS is hampered by measurement difficulties. It is quickly removed from the circulation by just one circuit through the body. Its blood concentration is the result of its rate of production and clearance. The body of work performed to investigate its involvement in ARDS is consequently very limited. The work of Wilson et al. is therefore of considerable interest showing that an inhibitor of bradykinin reduces mortality by 50% in endotoxic treated rats. But further work needs to be performed to fully evaluate the importance of bradykinin in ARDS and MSOF.

Section 1.

Protein mediators

1b. Cytokines

TNF/cachectin and interleukin.

Introduction

Cachectin was discovered during studies of rabbits infected with *Trypanosoma brucei* in an experimental model of cachexia, a condition associated with extreme weight loss, depletion of lean body mass and hypertriglyceridaemia (Kawakami and Cerami 1981).

Kawakami and Cerami (1981) showed that when mice were injected with endotoxin, a transferable serum factor causing lipoprotein lipase (LPL) suppression and lipaemia was produced. LPL is a membrane bound enzyme that normally mediates the clearance of circulating lipids. Lipopolysaccharide (LPS) or endotoxin resistant mice, used as controls, did not exhibit this response. However LPL suppression and lipaemia could be induced in endotoxin resistant animals when they were injected with the serum of endotoxin treated normal mice. This study showed that the active agent in promoting LPL suppression and lipaemia was blood borne.

This factor was isolated from the supernatant of endotoxin stimulated murine macrophages by Beutler et al. (1985) and named cachectin, because of its suspected role as the mediator of cachexia. Beutler et al. purified cachectin and found it to be a polypeptide hormone with a subunit size of approximately 17 kilodaltons.

A link between shock and tumour regression was observed in 1962 when O'Malley et al. reported that serum obtained from mice with endotoxic shock was capable of producing haemorrhagic necrosis of a tumour in other animals. This tumour necrosis factor (TNF) when injected into animals produced shock like symptoms, discussed below.

Beutler's research group (1985) established that TNF and cachectin were the same substance. Early in 1985, Beutler et al. noticed cachectin and

TNF produced an identical spectrum of bioactivities and were immunologically similar. A single injection of endotoxin into rabbits can produce about 1 mg of the hormone, far in excess of the 0.3 mMol Abe and co-workers (1985) found in serum during shock.

After the injection of endotoxin, TNF appears in the circulation within minutes, reaches a peak level after 2 hours and then rapidly declines in concentration until at 4 hours it is not detectable. Macrophages and monocytes do not continue to release TNF, even in the presence of persistent endotoxic stimulus. TNF secreting cells *in vitro* become refractory to further production and after subsequent endotoxic exposure produce a markedly attenuated secondary response. The half life of the hormone in the circulation is approximately 6 minutes.

TNF induces the release of another cytokine, interleukin 1 by monocytes, macrophages and endothelial cells. IL-1 is also known to activate neutrophils, stimulating their adhesion to endothelial cell surfaces and enhancing their phagocytic activity. Its effect on endothelial cells is to promote neutrophil adhesion (Nawroth et al. 1986).

Highly purified IL-1 was isolated in the late 1970s and extensive investigation focused on the biological activities of the peptide. It has recently become clear, through the use of pure IL-1 that many of these responses characteristically observed during endotoxaemia are not solely mediated by IL-1 but, rather, occur in response to cachectin /TNF. The TNF NH2 terminal amino acid sequence binds to specific receptors that are distinct from those of IL-1.

Neutrophil and endothelial activation by IL-1 and TNF alpha

In vitro studies indicate that both cytokines have an effect on neutrophils and endothelial cells, the two major participants in neutrophil emargination. Chemotaxis has been demonstrated in neutrophils and

monocytes with IL-1. Chemoattraction of lymphocytes by IL-1 has also been reported (Miossec et al. 1984).

IL-1 induces release of the specific granules from human neutrophils (Klamper et al. 1978), and TNF alpha induces the adhesion of neutrophils to endothelium *in vitro* (Gamble et al. 1985). The adhesion process is complex. When endothelium monolayers, *in vitro*, were stimulated by cytokines, no enhancement of adherence of neutrophils to the endothelial monolayer was observed, until Hoover et al. (1984) introduced leukotriene B4. Subsequently a number of investigators demonstrated that IL-1, TNF alpha and endotoxin have a similar effect and that the adhesion process can be blocked by inhibitors of protein synthesis (Bevilacqua et al. 1985, Schleimer and Rutledge 1986). The involvement of lipoxygenases in MSOF will be discussed later in this chapter, in section 2f.

Endothelial leukocyte adhesion molecule (ELAM)

Bevilacqua et al. (1987) demonstrated the existence of endothelial leukocyte adhesion molecules (ELAM), produced in response to stimulation by cytokines. They developed monoclonal antibodies against IL-1 and TNF treated endothelial monolayers. The antibody was found to recognise cytokine treated, but not untreated monolayers (Bevilacqua et al. 1985, 1987, Pober et al. 1986), suggesting that the monoclonal antibody was recognising the ELAM binding site. Peak neutrophil adhesion is observed after a 3-4 hour stimulation of the monolayer with the cytokines. The adhesion is blocked by cyclohexamine or actinomycin D. Unlike IL-1, TNF alpha can induce rapid adhesion of neutrophils to the endothelial monolayer, this effect is on the neutrophil and is protein synthesis and RNA independent. The neutrophil dependant and endothelium dependant mechanism of neutrophil adhesion require a functional CD11 to CD18 (CD cluster of differentiation) complex on the neutrophil membrane. Neutrophils of patients with congenital absence of the CD11 to CD18 complex or normal

neutrophils in which the complex is inhibited by a monoclonal antibody cannot be stimulated to adhere to endothelium (Pohlman et al. 1986, Harlan et al. 1985). The effect of TNF on neutrophil adhesion is maximally induced within 5 min and does not require protein or RNA synthesis. In contrast, maximum effects of TNF on human umbilical vein endothelial cells take 4 hours to develop and required RNA synthesis (Gamble et al. 1985). The rapidity of the effects suggests the induction of surface expression of adhesion promotion molecules.

Sources of TNF and IL-1.

Monocytes and macrophages were considered the main, if not unique source of IL-1 and TNF alpha. Although neutrophils secrete less than the monocyte on a per cell basis, they release a considerable amount considering the large number of these cells in the circulating, marginal and bone marrow pools and in acute inflammation exudate (Yoshinaga et al. 1987). Recently, it has been shown that IL-1 induces the synthesis of prostacyclin (Rossi et al. 1985, Albrightson et al. 1985), and stimulates platelet activation factor production by endothelial cells (Bussolino et al. 1986).

Endothelial cells treated with endotoxin can synthesise interleukin-1 (Stern et al. 1985, Miossec et al. 1986) 1 hour after incubation of endotoxin, so are both origin and target for IL-1.

Tissue receptors of TNF.

Beutler et al. (1985) showed that when labelled TNF is injected into mice it is concentrated by several tissues, shown in Table 33. Eight minutes after injection, liver, kidney, skin, lung and gastrointestinal tract together contain nearly 80% total extra vascular label. Liver, kidney, spleen and gastrointestinal tract have specific TNF receptors. When administration of radio-iodinated TNF is preceded by unlabelled TNF the amount of labelled

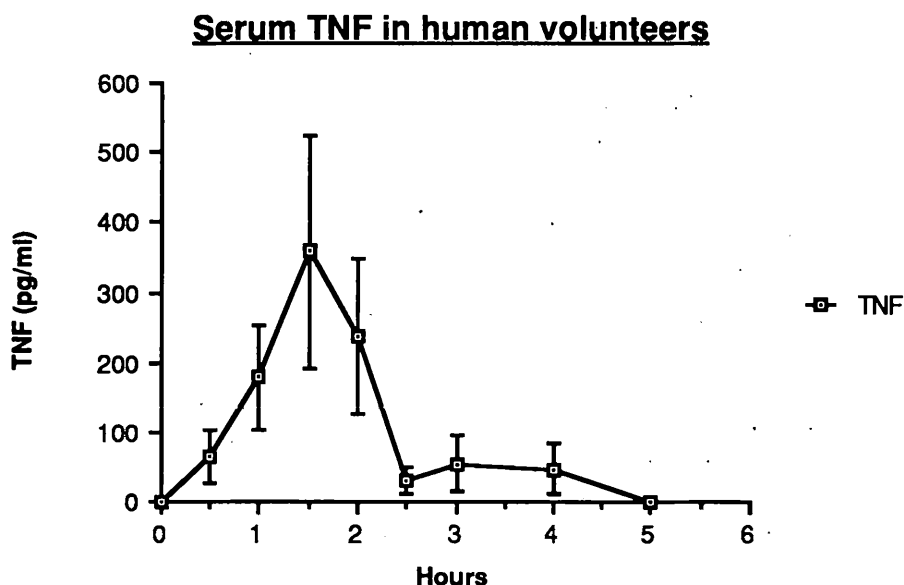
TNF is reduced. But the lung and skin receptors cannot be saturated in this way, suggesting that they are not so specific. The hormone is degraded rapidly in these tissues without formation of detectable intermediate products. Only 10% of the administered radioactivity was observed in the urine despite the fact that a large fraction of the injected hormone was recovered from the kidney itself.

Table 33. Fractional distribution of labelled TNF in tissue of mice, 8 minutes after injection. (Beutler et al. 1985)	
Organ	% of bound label
Liver	31
Skin	30
G.I. tract	8.8
Kidney	7.8
Lung	1.8
Spleen	0.9

TNF production *in vivo* in response to LPS or bacteria.

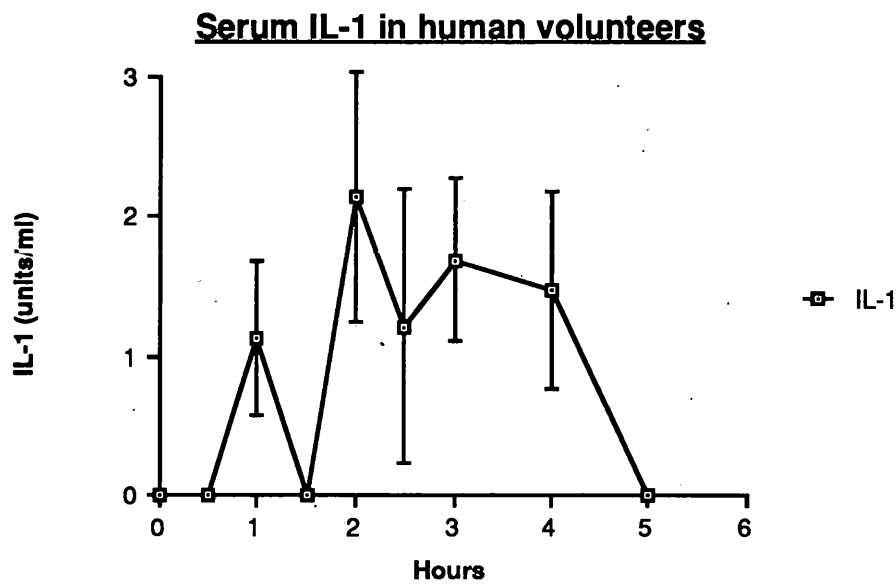
Hesse et al. (1988) gave bolus injections of endotoxin, 20 units/kg of body wt, to 4 volunteers. (It should be emphasised that 4 subjects is a very low number on which to base an hypothesis). On Figures 21-25 the endotoxin is given at time 0. Peak TNF levels of 358 ± 166 pg/ml in serum were observed within 1.5 hours of the challenge, falling to undetectable levels at 5 hours, seen in Figure 21.

Figure 21. Serum TNF concentrations in human volunteers, over 6 hours, challenged with 20 units/kg *E. coli* endotoxin. (Hesse et al. 1988)



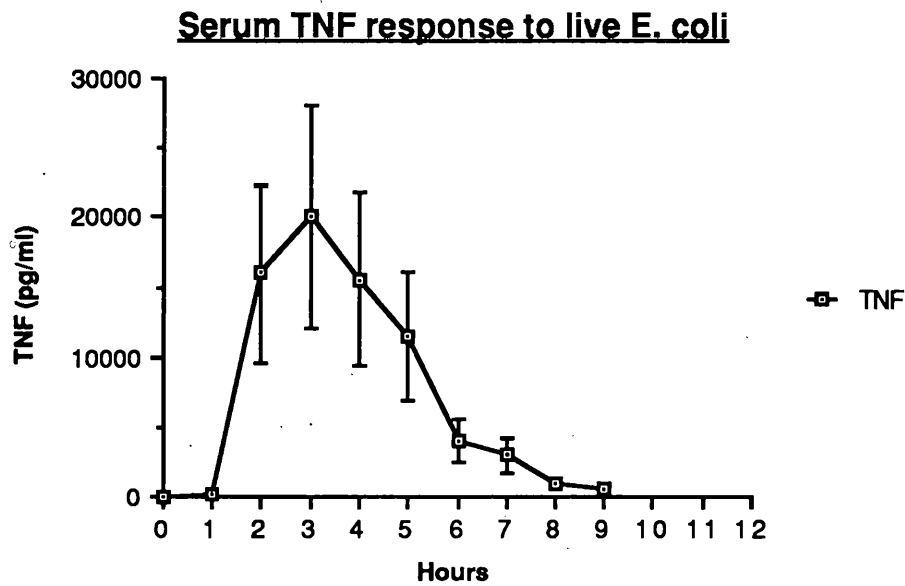
Peak serum IL-1 levels of 2.14 ± 0.89 u/ml were seen within 2 hours of challenge, falling to undetectable levels at 5 hours, as can be seen in Figure 22.

Figure 22. Serum interleukin-1 concentrations in human volunteers, over 6 hours, challenged with 20 units/kg *E. coli* endotoxin. (Hesse et al. 1988)



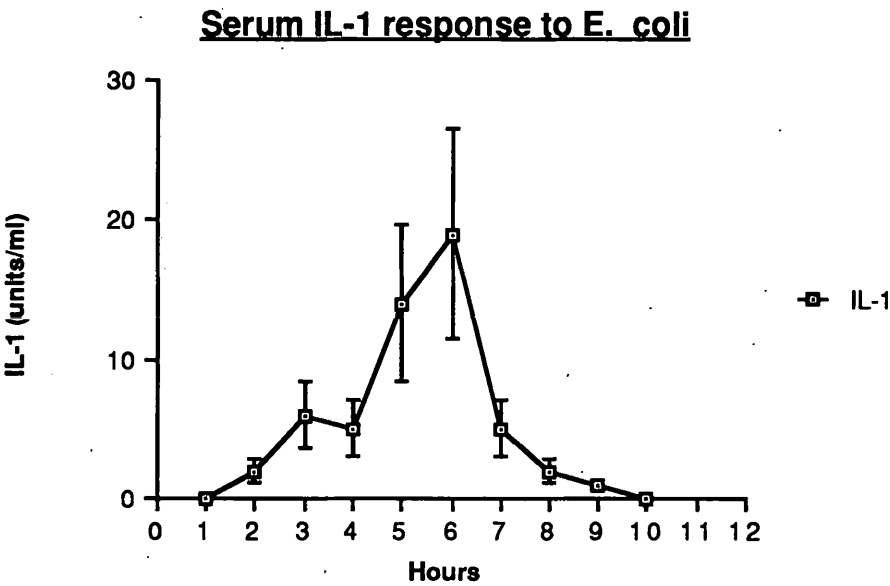
By contrast, the infusion of a lethal dose of live *E. coli* in to 4 baboons revealed peak serum TNF levels of $20,500 \pm 9890$ pg/ml within 1.5 hours of infusion, falling to undetectable levels at 9 hours, as can be seen in Figure 23.

Figure 23. Serum TNF concentrations, over 12 hours, in baboons challenged with a lethal dose of live *E. coli*. (Hesse et al. 1988)



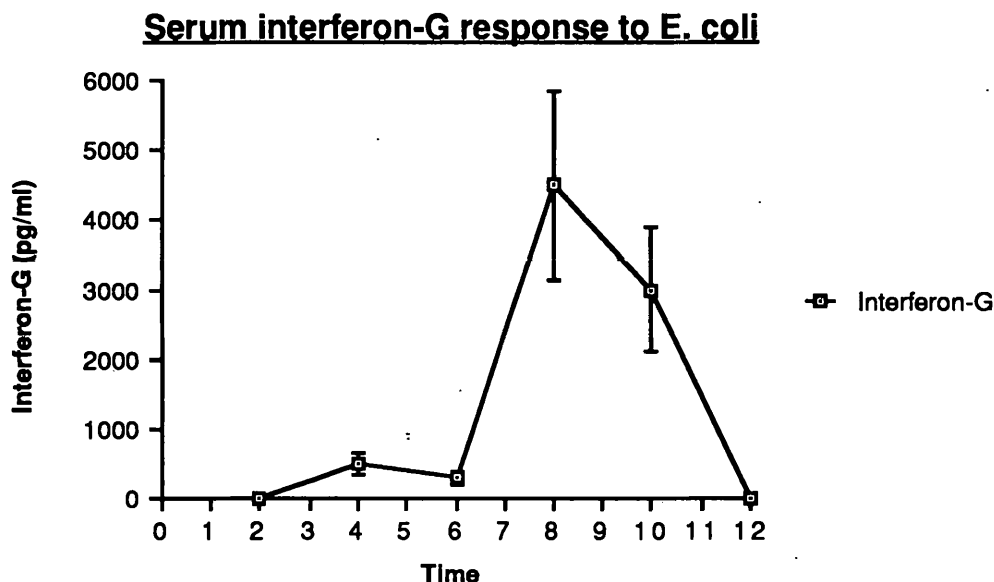
Peak IL-1 levels of 14.2 ± 10 u/ml, can be seen 3 hours after challenge, falling to undetectable levels at 10 hours, seen in Figure 24.

Figure 24. Serum interleukin-1 concentrations, over 12 hours, in baboons challenged with a lethal dose of live *E. coli*. (Hesse et al. 1988)



Interferon gamma levels reached a peak of 2.67 ± 1.66 ng/ml in baboon sera 8 hours after infusion, but was not detectable by 12 hours, seen in Figure 25. Gamma Interferon was not detected in the sera of humans.

Figure 25. Serum interferon-g concentrations, over 12 hours, in baboons challenged with a lethal dose of live *E. coli*. (Hesse et al. 1988)



These results suggest that the transient release of TNF followed by IL-1 and interferon gamma may participate in a cascade of events noted in overwhelming bacterial invasion. Although the time course of the cytokine appearance was similar in both models, lethality appeared to correlate only with its magnitude.

These data show that serum TNF levels of 300-400 pg/ml and IL-1 levels of 2-3 μ /ml are well tolerated in healthy normal volunteers, as they did not show any adverse symptoms of endotoxemia. Elevated circulating levels of TNF and IL-1 appear to be transient events in acute sepsis. Therefore the early TNF and IL-1 response to the infection may represent an important but transitory triggering signal in the host response to invasive infection. Interferon g production appeared to be a transient phenomenon reaching peak levels 8 hour after bacterial challenge in baboons. No interferon g was found in the serum of human volunteers challenged with endotoxin. These

data suggest that circulating levels of interferon γ may depend on the scope and duration of the initial bacterial insult or alternately on the magnitude of the cytokine response to bacterial stimuli.

Lymphotoxin, a product of activated T cells with a spectrum of biological activities similar to TNF, could not be detected in humans or baboons.

TNF stimulates endothelium or macrophages to produce IL-1. Although the infusion of IL-1 has been reported to induce fever and hypotension, unlike TNF, IL-1 does not appear to induce irreversible shock and death. Extremely low tissue levels of TNF and IL-1 are known to elicit several cellular responses in the presence of other cytokines. Many investigators have shown that interferon γ acts synergistically with TNF and IL-1 in several biological systems. Whether or not the delayed appearance of circulating interferon γ synergises the very low levels of TNF and so perpetuates the injurious bacterial state or alternately mediates the action of other cytokines in the lethal host response requires further investigation.

Shock in response to TNF infusion.

TNF was administered (Tracey et al. 1987) by infusion in two groups of beagle dogs at a lethal dose of 100 $\mu\text{g/kg}$ and sublethal dose of 10 $\mu\text{g/kg}$, seen in Table 34. The infusion produced serum TNF levels 1-50 nmol/l similar to those achieved after experimental endotoxaemia. The lethal response to TNF was characterised by progressive hypotension, shock and death within three hours. TNF infusion precipitated significant increases of plasma catecholamines in a dose dependant manner. TNF infused directly into the isolated hindlimb mediated reductions of skeletal muscle resting transmembrane potential and stimulated lactate production efflux.

Histopathological findings after the lethal dose of TNF showed gross abnormalities in the lung, kidneys and adrenal glands, with multiple

haemorrhagic lesions in the pulmonary parenchyma. The kidneys also contained multiple haemorrhagic foci and the adrenal glands were grossly necrotic. Leucocytes and thrombi were aggregated in the pulmonary vessels.

Acute tubular necrosis and acute interstitial inflammation was apparent throughout the kidneys. Haemorrhagic medullary necrosis was prominent in the adrenal glands.

Table 34. Haemodynamic and neurohormonal response of dogs to TNF infusion. (Tracey et al. 1987)

Time	MAP	PCWP	CO	SVR	AD	NorAD
Hrs	mmHg	mmHg	L/min	units	pg/ml	
TNF Dose 0 µg/kg						
-1	139	6	2.3	4626	33	223
0	128	6	1.8	5422		
1	123	5	1.7	5552		
2	133	5	1.8	5688	55	359
3	127	4	1.8	5466	57	273
TNF Dose 10 µg/kg						
-1	145	5	2.6	4307	36	307
0	143	6	2.1	5219		
1	113	4	2.1	4152		
2	130	3	2.3	4417	62	32
3	124	5	1.8	5288	145	565
TNF Dose 100 µg/kg						
-1	141	5	2.5	4320	58	282
0	113	1	1.8	4977		
1	98	0	1.9	4126		
2	93	3	1.5	4800	1148	1400
3	45	2	1.5	2293	1623	1982

MAP mean arterial blood pressure, PCWP pulmonary capillary wedge pressure (reflected left atrial pressure), CO cardiac output, SVR systemic vascular resistance, AD adrenaline, NorAD noradrenaline.

The falling PCWP, seen in Table 34, in the high dose group necessitated the administration of intravenous fluid at a rate which significantly exceeded the fluid requirement of the other two groups, showing that TNF participates in the development of tissue oedema and increased permeability oedema, typically seen during the development of shock. CO and SVR fell causing a fall in MAP. The reduction in SVR shows that systemic vasodilatation occurred, with a reduction in PCWP contributing to the development of the shock syndrome. Profound shock and falling blood pressure were associated with progressively falling cardiac output, in spite of markedly increased levels of circulating catecholamines.

In some studies by this group, the hind limb was isolated from the rest of the circulation of the dogs, by use of an extracorporeal circulation machine. When TNF was infused into the isolated hind limb lactate was released. The control dogs that had a placebo infusion did not produce lactate. These data suggest that TNF contributes to the development of a profound lactemia by an unknown mechanism. When TNF alone is infused, many of the features associated with shock are produced. These features show many similarities to those produced by the infusion of endotoxin.

Tracey et al. (1986) found similar results when they infused TNF into rats. They found histological damage to the lungs, kidney and G.I. tract and hypotension, metabolic acidosis, haemo-concentration and death within several hours.

Tracey et al. (1987) produced lethal bacteraemia in baboons, blocking the endogenous TNF by passive immunisation with monoclonal anti-TNF, shown in Table 35 below.

Table 35. Attenuation of haemodynamic responses to TNF infusion, by TNF antibodies, in baboons. (Tracey et al. 1987)						
Time	TNF	MAP	WBC	CO	AD	NorAD
Hrs	ng/ml	mmHg	x1000	L/min	pg/ml	
Controls-live <i>E. coli</i>						
-2	<0.04	120	13.2	2.2	165	382
2	16.7	65	1.1	2.8	973	2344
8	<0.04	0	1.2	0	3012	3411
Antibody 1 hour before infection						
-2	<0.04	120	9.3	2.4	129	958
2	1.2	110	1.0	3.0	1785	2591
8	<0.04	115	1.4	2.8	3202	3934
Antibody 2 hour before infection						
-2	<0.04	120	12.1	2.1	191	419
2	<0.04	110	3.6	2.4	550	491
8	<0.04	115	8.2	2.3	160	372

Baboons were given monoclonal anti-TNF intravenously, 1 and 2 hours before the LD₁₀₀ dose of live *E. coli*. Maximum plasma TNF levels of 22 ng/ml in the controls were observed for 1.5-2.5 hours following the *E. coli* infusion. Serum TNF fell from nanomolar concentrations to below detectable levels of 34 pg/ml, 4-6 hours after bacterial infusion. Control animals rapidly succumbed to the lethal effects of bacteraemia, with an acute decrease in both blood pressure and the PCWP and a decline in cardiac output, vital organ injury and anuric renal failure. There was a 2 fold increase in serum creatinine followed by death from pulmonary

oedema. These pathophysiological events are characteristic of lethal haemodynamic sepsis.

Passive immunisation of baboons with anti-TNF antibody infused 1 hour before bacterial challenge conferred beneficial cardiovascular effects, but not complete protection against critical organ injury. Although systemic vascular resistance fell, blood pressure was maintained by a compensatory increase in heart rate and cardiac output. Although acute cardiovascular collapse and shock were not observed serious renal injury did occur, a twofold increase of serum creatinine was seen at 8 hours with the development of anuria. Each of the baboons immunised 1 hour before bacterial challenge developed fatal pulmonary oedema.

The antibody when administered 2 hours before the *E. coli.* infusion was associated with maintaining normal blood pressure and preventing the development of shock. Vital organ dysfunction did not occur or renal failure develop. There was no evidence of pulmonary oedema. These results with TNF antibodies were unlikely to be due to increased bacterial clearance in the immunised animals or to bacteriocidal properties of the antibody solution. Antibody administration is associated with improved cardiac output, suggesting that the well known sepsis associated myocardial depression is also in part due to TNF.

The role of TNF as a mediator of endotoxic shock was further established by studies in which mice (Beutler et al. 1985) were immunised against the hormone and found to be protected against the lethal effects of endotoxin.

Prostaglandins and TNF.

Kettelhut et al. (1987) suggested that prostaglandins participate as second messengers of TNF induced systemic toxicity. After intravenous injection of human recombinant TNF (4 mg/g) into rats, all died within 2-4 hours. In 1 hour TNF caused a sharp fall in body temperature and a large

increase in plasma prostaglandin E2 levels. Blood glucose initially increased but then a profound hypoglycemia developed by 2 hours. The TNF treated animals developed cyanosis and severe metabolic acidosis. A single injection of the cyclo-oxygenase inhibitor indomethacin or ibuprofen before the TNF treatment completely prevented the rapid killing and reduced lethality by 70%.

A single dose of cyclo-oxygenase inhibitors (indomethacin or ibuprofen) prevented lethality, acidosis, hypothermia and biphasic changes of blood glucose concentration. These data suggest that prostaglandins are direct mediators of the cellular responses, seen during septic shock, that are triggered in part, by the appearance of TNF. It must be emphasised that there aren't any papers in the literature showing cyclo-oxygenase inhibitors have these effects in septic animal models of ARDS. This work suggests that there should be some doubt concerning the pivotal role of TNF in septicaemia.

Human TNF

Waage et al. (1986) measured TNF in serum from 23 cancer patients, 23 patients with bacteraemia and 25 normal controls. The serum from normal controls and cancer patients did not show the presence of TNF. Only 3 of the infected group showed the presence of TNF: two on admission but not later; the third patient developed bacteraemia in hospital and only showed an increased TNF on one day. Waage et al. (1986) does not describe whether the patients had shock, whether the TNF positive patients were in any way different to the other infected patients, or how many died. Waage et al. (1986) also showed one patient with TNF was infected with non-endotoxin producing Gram-positive pneumococci, suggesting that factors other than lipopolysaccharides may stimulate production of TNF.

Waage et al. (1987) looked at TNF serum concentrations in 79 patients on admission with meningococcal meningitis, septicaemia or both. The

information is summarised in Table 36, but does not give the plasma concentrations of TNF, only its presence or absence.

TNF was present in 18 of the patients, 10 of whom died. Thirteen of the patients with TNF had septic shock on admission, and hypotension developed in a further 4 of the TNF patients. He also showed a high correlation between TNF and granulocytopaenia. The patients with the highest level, greater than 0.1ng/ml eventually died.

Although Waage et al. (1987) has shown a strong correlation between the presence of TNF in the initial stages of shock and a fatal outcome, he (Waage et al. 1987) was unable to show the presence of TNF in the serum of shock patients after the initial phase. His findings that TNF concentrations in excess of 100 pg/ml are predictive of death throw some doubt on the importance of TNF measurements as a predictor of shock and outcome. Higher serum levels have been shown in non-lethal conditions. Human volunteers receiving *E.coli* endotoxin at sublethal doses had serum TNF concentrations of 350 pg/ml (Hesse et al. 1988). Scuderi (1986) showed that in patients with visceral *Leishmania* or malaria TNF levels of 188pg/ml were present in the serum.

Table 36. Association of serum TNF with clinical outcome of 79 septicaemic patients. (Waage et al. 1987)

Category on admission	Survived	Died	TNF (positive)
Positive meningitis with no hypotension or bruises.	26	0	2
Septicaemia with hypotension but no meningitis.	12	8	11
Septicaemia with hypotension and meningitis.	15	2	2
Septicaemia with or without meningitis with no hypotension or bruises.	15	1	3

Hypotension: < 100 mmHg

Conclusion.

Although TNF and IL-1 are important mediators in shock and MSOF, it is likely that TNF is only involved in the initial stages of the process. It should also be emphasised that serum levels do not necessarily reflect the level of TNF involvement. A more exact measurement of TNF activity might be the measurement of messenger RNA for TNF in monocytes.

Monoclonal anti-bodies to TNF, given after endotoxin infusion, do not prevent death, and even when given an hour before infusion, they do not prevent severe organ damage.

When cyclo-oxygenase inhibitors are given before TNF infusion, they considerably attenuate the haemodynamic, histological and biochemical changes. But cyclo-oxygenase inhibitors have little effect on animal models of ARDS and MSOF.

Section 1.**Protein mediators.****1c. Complement.****Introduction**

The complement system consists of at least 20 chemically and immunologically distinct serum proteins which are capable of interacting with each other, with antibodies, and with cell membranes. These interactions lead to the generation of complement activity. The biological sequelae of activation of this system range from lysis of a spectrum of different kinds of eukaryotic cells, bacteria and viruses to direct mediation of inflammatory processes. The individual proteins of this system are normally present in the circulation as functionally inactive precursor molecules. Together they comprise approximately 15% (w/w) of the plasma globulin fraction.

Biological actions of complement cleavage products.

Two peptides are principally involved in inflammation that are chemically distinct from each other. C3a is a peptide with a molecular weight of 8,900. C5a is larger, molecular weight 17,000 kD. C3a and C5a are also biologically distinct in that they react with different cellular receptors, since tissue rendered unresponsive to C3a will continue to respond to C5a, and vice versa. C5a also has chemotactic activity, for leucocytes. Binding C5a to leukocyte membranes, particularly neutrophils, causes migration of the cells towards the area of complement activation.

Complement activation via the classical and alternative pathways

The classical pathway is activated by antibody/antigen complexes, and is thought to be of relatively little importance in the development of MSOF. Activation of the alternative pathway occurs in the absence of antibodies, it occurs in response to such factors as bacterial polysaccharides.

Complement activation in the presence of leucocytes, leads to the release of leukocyte lysosomal enzymes. This is a specific reaction, as other compartments of the cell are not affected and lysis does not occur. The active principle in the clinical aspects of complement activation is C5a.

Complement activation in haemodialysis.

When haemodialysis was first introduced, it was associated with pulmonary dysfunction and hypoxemia and cardiopulmonary dysfunction, including angina pectoris, dispnoea, cardiac dysrhythmia and even sudden death (Hampers et al. 1973).

In 1968, Caplow and Gochinet (1969) discovered that sudden severe leucocytopaenia occurs in the first few minutes of haemodialysis. Although profound, this reduction in the number of leucocytes in the blood of humans, usually occurred for less than 30 minutes. Initially, the leucocytopaenia was presumed to be the result of brief sequestration of leucocytes in the cellophane coils of the the haemodialysis apparatus. However, when autologous plasma devoid of leucocytes was incubated with dialyser cellophane, and infused into dogs, leucocytopaenia also developed (Jenson et al. 1973). These results demonstrated that plasma incubated with dialysing cellophane, generates a factor producing sudden and dramatic changes in the behaviour of leucocytes.

Craddock et al. (1979) showed that leucocytopaenia was due to complement activation. This occurred when plasma contacted the cellophane membrane of the dialyser, via the alternative pathway. Pulmonary dysfunction was found in both patients and animals (Craddock et al. 1977). Leukocytes, predominantly neutrophils and monocytes were sequestered mainly in the lungs of patients (Craddock et al. 1979), undergoing dialysis. In rabbits leucostasis was accompanied by increased pulmonary lymph flow.

Neutrophil activation by complement.

Endotoxin and zymosan activate complement by the alternative pathway. Electron microscopy of neutrophils exposed to activated complement showed rapid surface changes including membrane spreading, pseudopod development and extrusion. These features suggested that such neutrophils might adhere more readily to the endothelium (Craddock et al. 1977).

Sachs et al. (1978) studied the mechanism by which the combination of activated complement and neutrophils, damaged the pulmonary endothelium. He exposed endothelial cells cultured from human umbilical veins to granulocytes activated by C5a. These granulocytes adhered to the endothelial cells and released toxic oxygen species such as superoxides and hydrogen peroxide. Substantial endothelial damage resulted, but he found that this damage could be prevented by the addition of the oxide detoxifier enzyme, superoxide dismutase. Neutrophils exposed to endotoxin also damaged the endothelial cells *in vitro*, a finding that seems particularly relevant to patients with sepsis and ARDS (Craddock et al. 1977).

Craddock et al. (1977) found that activated plasma complement will induce aggregation of human granulocytes. He thought the active component was C5a because anti-C5a could ablate this response but not anti-C3a antibodies. C5a fragments prepared by trypsinisation of purified C5a reproduced the aggregating activity of the whole activated plasma, whereas plasma from C5a deficient donors did not produce this aggregation. Granulocyte aggregation and activation was induced by either injecting zymosan or exposing plasma to cellophane.

Webster et al. (1982) investigated the action of C5a infusion, systemic complement activation by cobra venom factor (CVF) and intravascularly administered zymosan-activated (ZAP) rabbit plasma in rabbits. They were interested in measuring the degree of lung injury in these animals. They found that C5a, CVF and ZAP all caused an acute neutropaenia, that with

sequestration of neutrophils within the pulmonary vasculature. However no significant lung inflammation, as measured by neutrophil emigration from the pulmonary capillaries, or increased vascular permeability, occurred with any of the three stimuli. Only when these agents were combined with anaesthesia, surgical manipulation and intubation, did significant neutrophil emigration into alveoli occur, but there was no change in vascular permeability. One must conclude from these data, that complement activation alone is not enough to cause symptoms of ARDS. Additionally, they showed that pulmonary leucostasis can occur without pulmonary damage.

Cardiopulmonary by-pass activation of complement.

It has also been shown, that patients undergoing heart lung by-pass surgery, show what is called 'post pump syndrome,' similar to ARDS. Chenoweth et al. (1981) concluded, that the complement derived inflammatory mediators, C3a and C5a, produced during extracorporeal circulation, may contribute to the pathogenesis of this post by-pass syndrome. He measured levels of C3a and C5a, during cardio-pulmonary by-pass in 15 adults. Plasma levels of C3a were significantly elevated at the beginning of the procedure, and continued to increase steadily thereafter. At the end of the procedure, C3a levels were more than 5 times higher than the pre-operative levels. Plasma levels of C5a did not change significantly during cardio-pulmonary by-pass, instead, there was a significant neutrophilia during the by-pass, and significant transitory neutropaenia occurred when cardio-pulmonary circulation was re-established with partial by-pass. Chenoweth et al. (1981) found that incubation of blood with the nylon mesh liners of bubble oxygenators, as well as vigorous oxygenation of whole blood, promotes the activation of complement.

Anaphylatoxins.

The vaso-reactive products of complement activation are termed anaphylatoxins, since on injection they produce a syndrome similar to that seen in anaphylactic shock. This effect was shown as long ago as 1917 in humans, although they were ignorant of complement as the activator at that time, as complement was unknown (Noble and Kruph 1917).

The anaphylatoxins have been characterised as low molecular weight polypeptides released during activation of C3, C4, C5. These peptides termed C3a, C4a and C5a are characterised by the ability to enhance vascular permeability when injected subcutaneously and to release histamine from mast cells causing smooth muscle contraction. In addition C5a is a potent chemoattractor of neutrophils (Fernandez et al. 1978). It is significant that complement activation in humans with gram negative sepsis is associated with the development of shock and high mortality (Fernandez et al. 1978).

Neutropaenia.

Hosea et al. (1980) found that the infusion of activated C5 into guinea pigs increased alveolar capillary permeability to serum proteins, but rabbits made neutropaenic did not alter their capillary permeability. Activation of complement through C5a in the presence of neutrophils induces alterations in pulmonary alveolar capillary permeability.

Tvedten et al. (1985) found that acute lung injury was produced in mice by intravenous injection of cobra venom factor, an activator of C5. He also found that depletion of neutrophils and platelets resulted in marked reduction in the degree of lung injury. Lipoxigenase and thromboxane synthetase inhibitors afforded some protection against cobra venom factor induced acute lung injury, while cyclo-oxygenase inhibitors gave variable results. His data suggested that acute lung injury in mice following systemic activation of complement had an absolute requirement for C5a and

was dependant on the role of neutrophils as well as platelets and can be linked to the generation of lipoxygenases (Tvedten et al. 1985).

Tvedten et al. (1985) showed by transmission electron microscopy that after the injection of cobra venom factor and production of C5a, pulmonary vascular injury developed with extensive swelling of endothelial cells. These changes were associated with intravascular aggregation of platelets, neutrophils and fibrin and occlusion of pulmonary capillaries.

C5a desaturated arginine.

Larson (1980) compared C5a and C5a desaturated arginine (C5adesArg). Because C5a is rapidly converted to C5adesArg in vivo, he looked at fragments most effective in producing pulmonary inflammation. In rabbit C5adesArg consistently produced marked inflammation. This was characterised by neutrophil accumulation, oedema, haemorrhage, fibrin formation and damage to alveolar epithelium. The time course of the inflammatory reaction initiated by C5adesArg showed pulmonary vascular sequestration of neutrophils but no intra-alveolar migration 30 minutes after injection. After 2 hours interstitial and alveolar neutrophils were numerous with accumulation of neutrophils in the alveolae, increasing to a maximum at 6 hours. At 24 and 48 hours the predominant cells were mononuclear macrophages. In contrast, inhalation of C5a induced either no inflammation or milder more focal response than C5adesArg. Infusions of C5a produced little inflammation. A prolonged intrapulmonary infusion of C5a over 20 minutes in contrast to the bolus infusion of 1 minute, did initiate an inflammatory response which may reflect the conversion of C5a to C5adesArg in the lungs. His study suggested that intrapulmonary cleavage of C5 plays an important roll in the initiation of pulmonary inflammation (Larson et al. 1980).

The same group then looked at the effect of circulating C5a and C5adesArg in adult rabbits, using the labelled mediators I^{125} C5a or I^{125}

C5adesArg. C5adesArg persisted in the circulation longer than C5a. Inhalation caused an acute neutropaenia whereas with C5adesArg it was less severe but more prolonged. Changes were primarily seen in the highly vascularised organs, lung, spleen, liver and kidney. The time dependant accumulation was seen initially in the lung followed by the spleen, liver and kidney. Histological examination showed a marked increase in the number of neutrophils within the lung and spleen. Depletion of circulating neutrophils by nitrogen mustard pre-treatment in rabbits showed no change in the amount of labelled mediator (I^{125} C5a or I^{125} C5adesArg) bound in the lung. Whereas splenic accumulation of labelled mediator depended on the presence of neutrophils. These results indicate that C5a and C5adesArg are rapidly removed from the circulation. Specific accumulation in the highly vascularised organs and clearance from the circulation of more than 50% of the radio activity was seen within 2 minutes for both mediators i.e. . These studies show neutrophil dependant and independent mechanisms are involved in the removal of C5a and C5adesArg from the circulation. Binding of C5 fragments in the pulmonary vasculature may precede and then induce neutrophil sequestration. The aggregation of neutrophils in response to activated complement might contribute to the genesis of ARDS.

Hammerschmidt et al. (1980) looked at 61 patients at risk of developing ARDS. He found a significant correlation between the presence of complement and neutrophil aggregating activity with the development of ARDS. This correlation was also significant when patients with sepsis were excluded from analysis. When patients with ARDS were studied, 31 of 33 had abnormal plasma C5a levels on the day of diagnosis or during the following 72 hours. In contrast only 5 of the 28 high risk patients who did not develop ARDS had excess plasma C5a.

Complement activation in the development of ARDS.

It seems paradoxical that within 30 minutes of beginning infusion of complement, activated by zymosan, cobra venom factor, haemodialysis or leukophoresis, pulmonary dysfunction and increased pulmonary lymph flow were observed. Yet, circulating C5a in patients with adult respiratory distress syndrome may be detected hours or days before pulmonary dysfunction is noted. The acute pulmonary dysfunction that has been reported was mild and was detected by standard techniques. In contrast, the dysfunction in ARDS is severe. It is possible that this severe pulmonary dysfunction will be detected if sought in the hours preceding the development of the syndrome. Furthermore, neutrophils repeatedly exposed to activated complement become unresponsive to the chemotactic and aggregating effects of C5a. Thus with prolonged circulation of C5a, neutrophils may become insensitive to this complement component and may take longer to damage endothelium (Hammerschmit et al. 1978).

In 1984, Duchateau et al. studied 50 patients, 36 of whom developed ARDS, and found an early intense complement activity. These patients were at risk of ARDS because of multiple injuries, major abdominal surgery, acute pancreatitis, burns, or disseminated intravascular coagulation. The C3d fraction, a breakdown product from C3 activation, was measured as well as C3. Absolute levels of C3d were correlated with total C3 content of normal plasma; therefore the ratio of C3d to total C3 concentration (C3d:C3 ratio) was calculated in order to assess true *in vivo* complement consumption. Abnormal C3 consumption, measured by the C3d:C3 ratio, and elevated plasma C5a activity were associated with respectively, 84% and 86% of cases of ARDS. The C3d:C3 ratio was increased in 12 control patients after minor surgery. C5a activity was found only in patients at risk of ARDS. It was highly associated with conditions that predisposed to the development of ARDS but it did not show any consistent increase of correlation with the occurrence of ARDS. Sequential samples from both sides of the pulmonary

circulation showed initial pulmonary clearance followed by the release of C5a like activity. No simultaneous changes in C3 levels were found suggesting the possible presence of modulation factors. These observations suggested that other factors may influence the development of ARDS.

Similar findings of a lack of correlation between the presence of serum complement and the development of ARDS have been shown by Weinberg et al. in 1983 and Parsons et al. in 1989. These findings suggest that whatever its involvement, complement activation alone cannot account for the development of ARDS.

Reduction of complement activity.

Flick et al. (1986) studied the effects of reducing the total haemolytic complement activity in sheep by *Naja haja* cobra venom factor. They infused E. coli endotoxin into 5 normal sheep and 5 pretreated with *Naja haja* and measured lung lymph flow. They showed that complement reduction did not prevent lung injury caused by endotoxin and did not prevent the appearance in plasma and lung lymph of chemotactic activity. One must conclude that an intact complement system is not necessary for endotoxin-induced lung injury.

Conclusion.

Complement is an important blood-borne mediator of inflammation, stimulating neutrophils, endothelial cells and the arachidonic acid cascade. But the development of ARDS is not only dependant on complement activation. Complement infusion into animals does not produce ARDS. Reduction of the complement component does not prevent pulmonary damage in animal models of ARDS. Additionally patients with ARDS do not show consistent correlation with complement concentration.

Complement is most likely important in the development of ARDS when associated with other inflammatory mediators. Complement is one

part of the inflammatory response seen in Figure 19, but the other blood-borne mediators are associated with the development of ARDS in the absence of complement.

Section 2.

Lipid mediators of inflammation.

2a. Phospholipids.

2b. Phospholipase.

2c. Arachidonic acid.

2d. Platelet activating factor.

2e. Cyclo-oxygenases; Thromboxane and Prostacyclin.

2f. Lipoxygenases; Leukotrienes.

2a. Phospholipids.

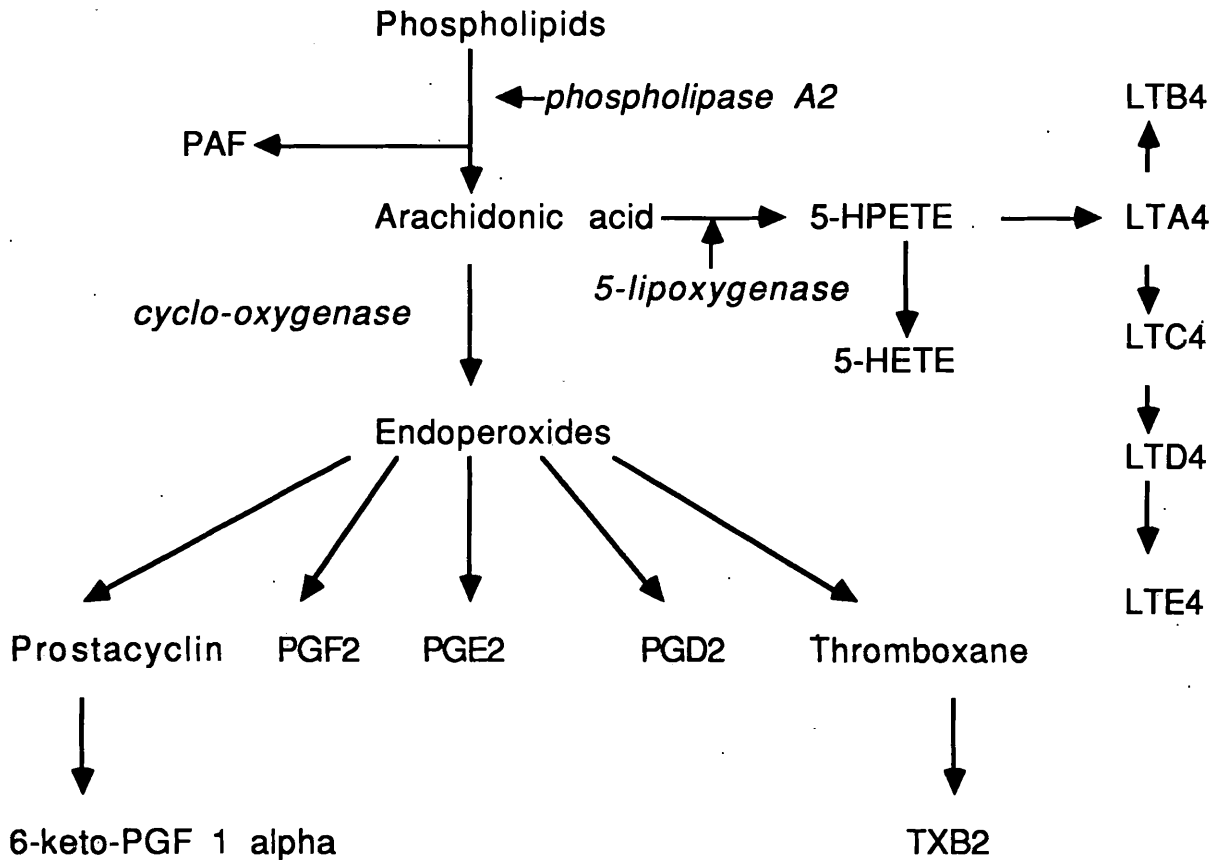
Introduction.

Prostaglandins (PG), thromboxane (TXA₂), prostacyclin (PGI) and leukotrienes (LT) collectively termed eicosanoids, are derived from the substrate arachidonic acid, a constituent of cell membrane phospholipid. A variety of inflammatory stimuli can directly or indirectly activate phospholipase A₂ or C, resulting in the release of arachidonic acid from specific phospholipid pools. (Flower and Blackwell 1976). Phospholipase A₂ is the rate limiting enzyme for the formation of eicosanoids. Once released, arachidonic acid is oxidatively metabolised via two major routes, the cyclo-oxygenase and lipoxygenase pathways, as can be seen in Figure 26. The enzyme fatty acid cyclo-oxygenase converts arachidonic acid to the unstable endoperoxide metabolites PGG₂ and PGH₂, which are metabolised enzymically to TXA₂, PG (PGD₂, PGE₂, PGF₂) and PGI₂. Although all eicosanoids are derived from arachidonic acid, the relative amounts of TXA₂ and PGI₂ vary with different cell types. These two cyclo-oxygenases are of particular interest because of their opposing haemodynamic and haematologic actions (Moncada and Vane 1979). Of the other cyclo-oxygenase products PGE₂ is a vasodilator, considerably less potent than PGI₂, and PGF₂ is a vasoconstrictor less potent than TXA₂ (Armstrong et al. 1978).

The other major pathway of arachidonic acid metabolism gives rise to the lipoxygenase products (Samuelson et al. 1979). Two classes of leukotrienes are derived from enzymatic conversion of the LTA₄ precursor: the sulphidopeptide leukotrienes, LTC₄, LTD₄ and LTE₄, and the nonpeptide metabolite LTB₄.

Prostaglandins, thromboxanes and leukotrienes can produce a variety of haemodynamic, haematologic and inflammatory actions that are characteristic of many features of circulatory shock. These eicosanoids have been increasingly implicated as significant pathophysiologic mediators in endotoxic and septic shock (Ball et al. 1986).

Figure 26. Lipid cascade in response to tissue damage in MSOF.

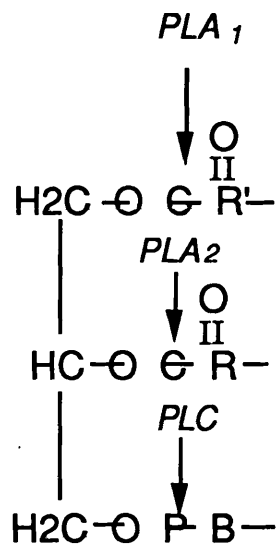


2b. Phospholipase.

Introduction

Phospholipases are a heterogeneous group of enzymes distributed throughout eukaryotic cells with molecular weights ranging from 5,000-100,000 kD depending on the organ and species in which they are found.

Figure 27. Structure of phosphatides and the action of phospholipase.



R is a fatty acid residue.

P is phosphate.

B is a choline, ethanolamine, inositol or serine group.

Phospholipids are composed of a 3-carbon glycerol backbone to which various functional groups are attached by an ester linkage to the glycerol alcohols. The 3 glycerol carbons are referred to as 1, 2 and 3, shown in Figure 27. Fatty acids are esterified in the 1 and 2 positions. The products of phospholipase A1 (PLA1) and phospholipase A2 (PLA2) hydrolysis are the 1 and 2 lysophosphatides, these include lysophosphatidic acid and platelet activation factor. Phospholipase C (PLA C) hydrolyses the glyceryl-phosphate bond on the 3 position to phosphoric acid giving phosphatidic acid, or more usually a phosphorylated base (i.e. inositol, choline, ethanolamine or serine) and a diacylglycerol, (diacylglycerol is a potent

stimulator of neutrophils that is independent of calcium ions). Arachidonic acid can be liberated from a phosphatide by PLA C if the enzyme is coupled with a diglyceride lipase. Coupled enzyme systems of this type have been described in platelets.

Thus PLA2 is an important regulatory enzyme for the entire eicosanoid cascade. PLA2 is found as two distinct types, either membrane-bound or soluble. Enzymes of the former group are strongly bound to the plasma, Golgi or mitochondrial membranes, generally requiring the presence of calcium ions and a neutral pH for optimal activity. The latter type of enzymes are soluble, found in cellular lysosomes, calcium independent and function at an optimal pH of 4. However soluble PLA2 with an optimum neutral pH and calcium dependence has been shown to be secreted during activation of phagocytes.

It is possible that the membrane bound enzyme can be stimulated when certain receptors on the cell surface are occupied. This could explain why certain agents such as bradykinin or an antigen-antibody combination can cause cells to generate large amounts of prostaglandins.

Clinical significance of serum PLA2 activity.

Vadas (1984) looked at serum PLA2 activity in patients suffering gram negative septic shock, non-septic shocked patients during the hypotensive phase, and normal volunteers.

All septic shock patients had elevated serum PLA2 activity as high as 434% compared to non-septic shock levels, and sixteen fold compared to control levels. But those patients with ARDS had a serum PLA activity 20 fold greater than controls, and 70% greater than septic non-ARDS patients. Patients with the highest levels of circulating PLA2 appear to be at greater risk of developing ARDS. Those patients surviving the hypotensive episode, showed decreasing phospholipase A2 levels, returning to baseline during their convalescence.

Vadas (1984) found that the PLA2 activity had an optimum pH of 7.5. This pH is typical of the membrane bound form, unlike the lysosomal form released on the degranulation of phagocytes, which has an optimum pH of 4.0. Vadas (1984) suggested that this biochemical characterisation implicated macrophages of the reticulo-endothelial system as the main source of this enzyme.

The administration of PLA2 is associated with hypotension. The intravenous injection of PLA2 purified from snake venom causes a rapid and profound drop in mean arterial blood pressure in dogs. Similarly PLA2 isolated from honey bee venom caused a marked hypotensive response in cats, dogs and rats. Vadas also showed that the intravenous administration of plasma from shocked rabbits thought to contain PLA2, produced a profound and sustained drop in MABP when injected into other normal rabbits. This hypotension was blocked by treatment with the phospholipase A2 inhibitor P-Bromophenylacetyl bromide.

Steroids have been shown to attenuate the severity of clinical Gram negative septic shock or experimental endotoxic shock. The mechanism of action of steroids is thought to lie in their ability to induce the synthesis of a protein inhibitor of soluble PLA2, termed lipomodulin or macrocortin. Alveolar macrophages are sources of PLA2 that can modify pulmonary surfactant. Further stimulation of reticulo-endothelial system macrophages may further enhance the secretion of this enzyme playing an important role in promoting tissue damage.

Vallee (1979) determined that the inhibition of PLA2 activity by non-steroidal anti-inflammatory drugs (NSAID) resulted in protection against inflammation and platelet aggregation. He used the PLA2 inhibitors, mepacrine, P-bromophenyl bromide and papaverine to assess their ability to prevent or decrease the release of arachidonic acid from platelets in the presence of thrombin. While the three agents could not all be considered sole PLA2 inhibitors, they all reduced the release of arachidonic acid from

platelets. BPB847 and CB874 were also used as they are direct inhibitors of PLA2 since they inhibit the *in vitro* hydrolysis of phospholipids by PLA2.

Vallee (1979) injected carageenan into the paws of rats treated with a drug or placebo. Measurements were taken by plethysmography after 2, 3 and 5 hours to gauge the intensity of the resulting oedema. Papaverine did not produce much inhibition, but mepacrine and the two numbered inhibitors gave considerable inhibition, and only 20% inflammation occurred. Mepacrine and the two numbered inhibitors caused inhibition of platelet aggregation when stimulated by thrombin. PLA2 inhibition reduced inflammation when stimulated by carageenan, showing its importance in the development of inflammation. It is assumed that this inhibition would prevent arachidonic acid release, lysophosphatide production and eicosanoid synthesis.

2c. Arachidonic acid.

Introduction

In man, the precursors of AA are usually ingested as dietary linoleic acid, a constituent in many meats. It is then esterified as a component of the phospholipid of the cell membrane or is found in ester linkage in other complex lipids.

AA is released from membrane phospholipids by the action of PLA₂. The concentration of free AA is low, and it is believed that endogenous biosynthesis of prostaglandins and related compounds depends on the PLA₂ release of precursor acid from cellular phospholipid stores.

Once released, AA is rapidly metabolised to oxygenated products by two distinct enzymic mechanisms, a cyclo-oxygenase and a lipoxygenase system. Both pathways of the arachidonic acid cascade may be activated by numerous mediators and appear closely linked to the pathophysiology of septic shock.

Eicosanoid substrate

Most mammalian cells generate eicosanoids in response to a wide variety of physiological, pharmacological or pathological stimuli and, since these compounds are not stored within cells, biosynthesis must immediately precede release. The substrate, usually arachidonic acid, must be in a non-esterified form, but the intracellular level of free precursor acid is extremely low. Although precursor fatty acids could arise from the hydrolytic cleavage of a number of intracellular lipid pools, eicosanoid release follows so rapidly on stimulation, that hydrolysis of dietary lipid is too slow to constitute a viable mechanism. Most cells and tissue types use phospholipids to produce AA.

2d. Platelet activating factor.

Introduction

The term platelet activating factor (PAF) proposed by Benveniste et al. (1973) originally defined the most prominent biological activity of a new class of phospholipids released from basophils by immune (IgE) stimuli. The chemical nature of these phospholipids has been identified as 1-O-alkyl-2-acetyl-sn-glycerol-3-phosphatidylcholine (Demopulos et al. 1979). This group of compounds have far more diverse actions than the stimulation of platelets. Endothelial cells and neutrophils are stimulated by PAF (Snyder 1985).

This vasoactive phospholipid produces severe hypotension, shock and death when infused into guinea pigs, rats, rabbits, dogs and pigs (Braquet et al. 1987). It is thought to be the single most potent agent to cause shock when administered systemically. Shock syndrome produced by PAF involves several mechanisms and sites of action.

First, the pulmonary circulation is affected by an increase in pulmonary vascular resistance and bronchoconstriction, both of which contribute to the development of hypoxemia, with the increase in pulmonary vascular resistance contributing to right heart failure (Bessin et al. 1983). Secondly, the heart is also affected by reduction in cardiac output due to coronary constriction (Feuerstein et al. 1983), reduction in myocardial contractility (Benveniste et al. 1983), and reduced cardiac preload. However some controversy exists as to whether PAF directly affects the myocardium or whether reduction in oxygen transport to the myocardium leads to a secondary effect, stemming from coronary vasoconstriction. Also, the PAF induced coronary vaso-constriction and cardiac failure seem to be mediated, at least in part by TXA₂, since much of the cardiac effect of PAF can be blocked by the prostaglandin inhibitor indomethacin (Feuerstein et al. 1983). Third, the peripheral circulation is affected by vasodilatation which might also contribute to the hypotensive effect of PAF. The vasodilation effect of

PAF is not mediated by eicosanoids (Feuerstein et al. 1983). Fourth, there is an increase in vascular permeability with extravasation of plasma into the tissue, contributing to the reduction in cardiac output and causing pulmonary oedema (Bessin et al. 1983). Fifthly, leucocytes and platelets are activated, primarily in the lungs and liver, contributing to endothelial damage and microthrombi in these organs. Finally PAF stimulates neutrophils and endothelium to produce secondary inflammatory mediators such as leukotrienes (Voelkel et al. 1982), thus confusing their direct effect on physiological and haemodynamic parameters.

Doses of 3 nmol/kg of PAF (a very low dose) reduce blood pressure and flow in rat mesentery, kidney and hind limbs. The blood pressure normally rises a short time after the infusion of PAF is stopped, but the blood flow in these organs remains low (Feuerstein and Hallenbeck 1987). Although infusion of PAF can cause the shocked state in animals, only a small number of studies have been performed on infection patients or animals to measure PAF levels. Elevated PAF levels were found in rats infused with *E. coli* endotoxin (Doebbler et al. 1985). PAF was also found to be released by macrophages and spleen lymphocytes in rats suffering from bacterial peritonitis (Inarrea et al. 1985). Antagonists of PAF such as kadsurenone, CV 3988, and BN 52021 have been shown to reverse hypotension (Doebbler et al. 1985), and increase survival in rats challenged with endotoxin.

2e. Cyclo-oxygenases; Thromboxane and Prostacyclin.

Introduction

TXA₂ has a very short half life, of 30 seconds at 37°C and pH 7.4, therefore TXB₂, a stable and virtually nonactive metabolite of TXA₂ is used to monitor TXA₂ levels. Several lines of evidence support a role for TXA₂ as a mediator of shock. Elevated plasma and lymph levels of TXB₂ are consistently found in a variety of animal shock models, such as endotoxic (Cook et al. 1980), traumatic (Hock and Lefer 1984) or haemorrhagic (Feuerstein and Hallenbeck 1987). Moreover, increased plasma levels of TXB₂ in humans suffering from severe septic shock were also reported (Reines et al. 1982).

Two major pathophysiological processes can be attributed to TXA₂, First, it is a potent vasoconstrictor of small and large blood vessels; second, it is an extremely potent aggregating factor of neutrophils and platelets. These two primary actions of TXA₂ are believed to act together to interfere with organ blood flow and promote ischaemia in several shock states.

Antagonists or synthesis inhibitors of TXA₂ also demonstrate its pathophysiology in various shocked states. The receptor antagonist EP 092 blocks the development of pulmonary hypertension in endotoxemia (Armstrong et al. 1985). However the TXA₂ antagonists are also PGH₂ antagonists, therefore it is not yet possible to evaluate the relative roles of PGH₂ and TXA₂ in shock, although TXA₂ synthesis inhibitors have therapeutic efficacy in several shock and trauma models. UK 37248 is effective in rat endotoxic shock (Halushka et al. 1983) and U 63,557A is protective in rat trauma (Reines et al. 1982). However it must be emphasised that TXA₂ inhibition fails to modify the outcome of gram negative septic shock (Fletcher et al. 1983). Inhibitors also act by increasing the availability of endoperoxides for the PGI₂ synthetase pathway to produce more PGI₂ (Casey et al. 1982).

Biosynthesis of thromboxane has been described in a range of tissues and cells including platelets, pulmonary endothelium, gastric mucosa, kidney, fibroblasts and leucocytes. Although it has recently been shown that all vascular endothelium can produce TXA₂, platelet and the action of thromboxane synthetase in platelets has been the most studied.

Prostacyclin, has a half life of 3 minutes at 37°C centigrade and pH 7.5, activated by the enzyme prostacyclin synthetase. It has been shown to be a major cyclo-oxygenase pathway metabolite of arachidonic acid in vascular tissue. Recent data from bovine aorta (Feuerstein and Hallenbeck 1987) shows that the enzyme responsible for the formation of PGI₂, prostacyclin synthetase, is found principally in endothelium and smooth muscle cells, although, the level of cyclo-oxygenase activity is several times higher in the endothelium. Histamine and bradykinin stimulate prostacyclin biosynthesis by human umbilical endothelial cells, whereas agents such as angiotensin 2, vasopressins, and adrenalin were without effect.

Thromboxane and prostacyclin exhibit a reciprocal relationship in blood capillaries. TXA₂ acts as a positive feedback to increase platelet and neutrophil aggregation and is a potent vasoconstrictor and lysosomal membrane destabiliser. Prostacyclin is a vasodilator and prevents platelet and neutrophil aggregation and causes their disaggregation. The TXA₂/PGI₂ balance is disturbed when endothelial cells are disrupted. Suppression of PGI₂ and augmentation of TXA₂ synthesis has been demonstrated in rabbit septic shock (Carmona et al. 1984).

Lung lymph was examined for prostanoids (Adams and Traber 1982) in sheep infused with endotoxin to produce ARDS. Thromboxane B₂ and 6-keto-F₁, were both elevated, but pretreatment with Ibuprofen abolished these early increases and prevented the resulting pulmonary hypertension. Prostaglandins could also be reduced by depleting the animal of their neutrophils (Huttemeier et al. 1982). It appears that in response to endotoxin, neutrophils release TXA₂, causing venoconstriction. The

microvascular hydrostatic pressure is raised, causing transvascular fluid movement and an elevation in lung lymph flow. The importance of PGI₂ is unclear, although PGI₂ antagonises most of the actions of TXA₂, it may not necessarily provide a protective effect.

Dupont et al. (1987) found a significant production of prostanoids in ARDS patients particularly those with sepsis. They found a disturbance in the balance of the prostacyclin and thromboxane relationship. Thromboxane and prostacyclin were measured in 84 patients at risk of developing ARDS, 44 patients following multiple trauma, 29 abdominal surgery and 11 with acute pancreatitis. ARDS developed in 49 patients, as can be seen in Table 37.

Table 37. Diagnostic distribution of 84 patients at risk of developing ARDS. (Dupont et al. 1987)		
Diagnosis	Patient number	ARDS patients
Multiple injuries	44	26
Abdominal surgery	29	14
Acute surgery	11	9
Total	84	49

Plasma thromboxane and prostacyclin levels can be seen in Table 38. High thromboxane levels were found in 52 of the patients. The median values of TXA₂ were 360 pg/ml in multiple trauma, 250 pg/ml in abdominal surgery and 410 pg/ml in acute pancreatitis. The median TXA₂ concentration was 575 pg/ml in patients developing ARDS, significantly higher than the 140pg/ml in those without this development. Median values of prostacyclin were 55 pg/ml in multiple injured, 25 pg/ml in abdominal surgery and 120 pg/ml in acute pancreatitis. The median PGI values was 122 pg/ ml in ARDS patients significantly higher than the 25pg/ml in non-ARDS patients.

High TXA2 and PGI values were particularly related to sepsis in abdominal surgery patients and were significantly raised.

Table 38. Plasma concentration of thromboxane and prostacyclin in 84 patients, in 3 diagnostic groups, at risk of developing ARDS. (Dupont et al. 1987)

Diagnosis	Thromboxane pg/ml	Prostacyclin pg/ml
Multiple injuries	60	55
Abdominal surgery	250	25
Acute surgery	410	120
ARDS patients	575	122
Non-ARDS patients	140	25

In multiple injured patients they were also significantly raised. No relationship could be established between abnormal TXA2 or PGI values and death. High TXA2 values often persisted for several days and were observed particularly at the time ARDS diagnostic criteria were fulfilled. An imbalance between TXA2 and PGI was observed. PGI values were always lower than TXA2 values and did not persist more than 24 hours.

Huttemeier (1982) examined TXA2 and PGI levels in sheep infused with *E. coli* endotoxin. Group 1 were control sheep, group 2 were rendered leucopaenic by nitrogen mustard, and group 3 received ibuprofen before the endotoxin infusion. The control sheep showed an increase in pulmonary vascular resistance and a high level of thromboxane release. The second group showed only half the increase in pulmonary vascular resistance and thromboxane levels. The third group showed only minimal increases of pulmonary artery pressure and pulmonary vascular resistance. Prostacyclin, on the other hand showed a 10 fold increase in group 1. But in group 2 and 3 there was no change. The production of prostacyclin seems to be dependant on the presence of leucocytes. In group 3 the prostaglandin

inhibition prevented the release of prostacyclin. Neutrophils appear to be unnecessary for the production of thromboxane when stimulated by endotoxin. Thromboxane, it should be emphasised, can be produced by the pulmonary endothelium, without the involvement of platelets.

There is varied production of eicosanoids by the endothelium of differing vital organs, explaining some of the regional blood flow variations occurring in shock. Cook et al. (1982) in 1982 showed that the hepatosplanchnic circulation is a region of increased eicosanoid production during endotoxemia in the rat. Venous TXB₂ and i6-keto-PGE₁ levels are increased relative to arterial levels, with the highest levels occurring in the portal or hepatic vein. High portal TXB₂ during endotoxemia correlates temporally with reduced splanchnic blood flow and small bowel infarction (Halushka et al. 1983, Frolich et al. 1980, Tempel et al. 1986).

2f. Lipoxygenases; Leukotrienes

Introduction.

The first animal lipoxygenases were not described until 1974, and it is only in the last ten years that they have been recognised as sources of biologically important molecules. Studies with fatty acids such as arachidonic acid or eicosapentanoic acid indicated the presence of a family of biologically important molecules which are derived from individual fatty acids.

Samuelson et al. (1983) introduced the term leukotriene in 1983 because of their initial discovery in leucocytes and their conjugated triene structure, to describe the compounds produced by this metabolic pathway. A subscript was used to describe the total number of double bonds in the molecule.

Production of leukotrienes in acute anaphylaxis is considered to play a major role in the cardio-respiratory derangements that lead to shock and death (Samuelson 1983). But the role of LT in other forms of shock such as sepsis, trauma, or haemorrhage is still unclear. This is primarily due to their rapid elimination from the circulation making the assay in biological tissues or fluids unreliable. Leukotriene metabolites must be measured, therefore in bile, urine or through bronchial lavage fluid.

Some recent evidence supports the generation of LT in burns and trauma by following the levels of LT in the bile. Large increments of LTE_4 and N-acetyl- LTE_4 are found in the plasma and bile of rats exposed to various traumatic injuries such as burns, bone fracture, abdominal surgery (Denzlinger et al. 1985) and endotoxemia (Hagman et al. 1985). Leukotrienes are considered to be rapidly cleared from the blood, and metabolised by the liver and excreted in the bile as nonactive metabolites. Recent experiments indicate that LTs have an enterohepatic cycle (Denzlinger et al. 1986) in species where N-acetyl- LTE_4 is the major LT metabolite to re-enter the circulation from the gut, although little biological

activity can be anticipated, since N-acetyl-LTE₄, is less than 1% as effective as LTE₄.

Leukotrienes have been shown to have effects on the cardio-respiratory system, the blood vessels and the microcirculation (Denzlinger et al. 1985). The principal pathophysiological actions of LT include, reduction in cardiac output, constriction of the blood vessels and reduction in organ blood flow, bronchoconstriction, reduced lung compliance and increased pulmonary vascular resistance (Piper 1982). An increase in post-capillary venular permeability and venoconstriction that leads to a pronounced plasma extravasation and reduction in blood volume (Piper 1982) is also observed. LT compounds have also been shown to aggregate and activate platelets, leucocytes and macrophages releasing their inflammatory mediators and contributing to the development of circulatory shock. This process also leads to the formation of microthrombi in the lungs.

Summary

Chapter 1 is a broad introduction to ARDS, showing the clinical picture and scientific background.

Chapter 2 describes the historic background and brings together the differing causes of ARDS. Most deaths medically described from ARDS were caused by sepsis, but with the improvement in medical practice, the condition was seen in traumatised but non-septic patients who would have previously died from their original condition such as trauma, burns or major abdominal surgery.

The Vietnam war allowed a comprehensive study of trauma victims who had immediate access to sophisticated medical treatment, who would previously have died shortly after injury. These studies showed that even when patients were treated quickly with volume replacement therapy, renal dialysis and mechanical ventilation, they still died, but not from their original injury. The lungs were normal in casualties with non-thoracic injuries who died within 8 hours of injury, but heavily congested in those who died later. All subjects with tissue trauma had some degree of pulmonary dysfunction, rarely associated with infection. This dysfunction appears to be a pulmonary response to tissue trauma.

This chapter additionally shows that ARDS is rarely seen without MSOF. Most patients die from organ failure or circulatory collapse, not hypoxemia. ARDS is only the more obvious pulmonary damage that occurs in association with other less clinically obvious organ dysfunction.

An analysis of the causes of MSOF suggests that it occurs as a result of a whole body inflammatory reaction. Any condition that produces a substantial inflammatory reaction can cause MSOF to develop. A better name for MSOF would be severe inflammatory cascade activation or SICA.

Chapter 3 looks at the major causes of MSOF and shows the common factors among them. Section 1 deals with sepsis. It shows that there is considerable confusion concerning the concept of sepsis among the medical

establishment. A number of terms in common usage such as septicaemia, septic syndrome, septic shock and bacteraemia are ambiguous, overlapping, imprecise and vague.

Sepsis is typically diagnosed in a patient by the symptoms of their condition, not the presence of micro-organisms. Micro-organisms are assumed to be present if the symptoms fit the clinical picture. The concept of septic syndrome has been established to describe the presence of symptoms in a patient, but the absence of a positive culture.

The science of microbiology seems to study the behaviour of a micro-organism in infections such as typhoid or cholera, but not their clinical implications in sepsis and MSOF. The medical fraternity is principally concerned with identifying the micro-organism so that the most appropriate antibiotic can be used in its treatment. Although antibiotics can cure bacteraemia, they make little impact on the high mortality of patients with septicaemia.

The symptoms seen in the septic syndrome are the patient's response to tissue injury. Although infection can cause that injury, there are many other causes of tissue injury not involved with micro-organisms. The underlying condition must be treated or the focus of inflammatory response removed if the patient is to have any chance of survival.

A similar pattern is seen in the treatment of severe trauma. Attention is focused on treating the symptoms of fracture and resetting the bone. Most trauma results in pulmonary dysfunction, usually at a subclinical level. Only when organ damage becomes evident is therapy initiated. There is a preference to respond only to a patient's deterioration, not to initiate therapy preventing such deterioration. There is little attempt to predict and prevent the development of MSOF in multi-trauma patients.

Injury to the quadriceps and femur is associated with the highest incidence of complications and MSOF. The mechanism by which MSOF develops in such patients involves severe inflammatory cascade activation in

response to tissue contusion. Substantial injury to the large muscle mass of the quadriceps allows a profound development of the inflammatory process. In most cases the high energy required to break the femur guarantees considerable muscle contusion. The bone must be reset and the tissue debrided within 24 hours. The use of traction and plaster should be abandoned as it delays therapy designed to reduce inflammatory cascade activation.

These two causes of MSOF are responses to a specific incident such as the start of an infection or the clinical developments after a car crash. But there is a third factor that is more insidious, that of tissue damage due to oxygen deprivation.

Oxygen deprivation and reduced energy metabolism are currently thought to be central to the development of MSOF. Tissue damage due to oxygen deprivation manifests itself at several levels. There is an increased metabolic rate due to sepsis, trauma, burns and other predisposing conditions. When this increased rate is limited by an inadequate oxygen supply then a relative tissue hypoxemia occurs.

In sepsis syndrome there may be a high tissue oxygen delivery, but the distribution of blood to organs and within the organs becomes inappropriate. Additionally a high oxygen delivery may be maintained in organs with a low oxygen extraction, such as the kidney or skin, at the expense of organs with a high oxygen extraction such as the brain and heart. The organ also loses the ability to distribute flow equally within itself, resulting in tissue hypoxemia.

Tissue hypoxemia allows the production of xanthine oxidase, an enzyme that allows the conversion of oxygen into super oxides. These super oxides can cause local tissue damage. It has also been shown that in septic syndrome the production of ATP is impaired due to mitochondrial derangement and dislocation of oxidative phosphorylation. Pyruvate dehydrogenase is inactivated, preventing the entry of pyruvate into the

mitochondria. Inadequate pyruvate concentrations in the mitochondria reduce the production of acetyl co-A, needed in the Krebs cycle.

Chapter 4 reflects on the histological changes seen by electron microscopy. These studies have chiefly involved the examination of lung ultrastructure in humans with ARDS and in animal models.

The acute changes in the lungs, seen up to 3 days after injury are: capillary leucostasis, endothelial swelling and disruption, interstitial oedema, epithelial vacuolation and disruption and alveolar oedema. The chronic changes are those of repair, seen up to 3 weeks after injury. They include fibrin deposition, proliferation of type two pneumocytes and the development of an alveolar hyaline membrane.

Several animal, but no human studies, have been performed on other organs. Ultrastructural studies of the liver and spleen in animals has shown profound leucostasis. Liver studies have shown swelling of the sinusoidal endothelium and Kupffer cells, and occlusion of the sinusoidal lumen. But there is an absence of leucostasis in the heart, kidney and muscle capillaries. There are few reports about the condition of the endothelium in these organs. This may prove to be an important area of research. If there is endothelial swelling in these leucostasis free organs, then the damage will be due to some as yet unknown factor.

Little work has been done on the ultrastructural changes of cell organelles such as mitochondria or endoplasmic reticulum in MSOF. It is important to know the effect of the inflammatory reaction on these organelles. It is possible that damage to these organelles causes many of the changes seen in MSOF. Liver, kidney, heart and muscle are of particular interest and further study, especially in humans, is needed.

Chapter 5 is concerned with the behaviour of inflammatory cells, especially neutrophils. They are closely involved in the process of tissue damage, especially in the lungs liver and spleen. In these organs tissue damage is seen in close association with the inflammatory cells. It is

difficult to establish why they aggregate in these organs, although it is possible that the reticulo-endothelial system is clearing the activated neutrophils from the circulation.

Pulmonary damage does occur in patients with a very low circulating neutrophil count, although its severity increases with any rise in blood neutrophil count. Tissue damage has been seen in animal models of ARDS that have been rendered leukocytopenic. Additionally, pulmonary leucostasis has been demonstrated in animals in the absence of tissue damage. Neutrophils are undoubtedly important in causing organ damage in MSOF, but pulmonary damage occurs in the absence of neutrophils and they are not responsible for the failure of the heart and kidney seen in this condition.

Chapter 6 is concerned with the chemical mediators involved in whole body inflammation. They are divided into two groups, the protein and the lipid mediators. The diagram on page 148 shows that there are many such mediators and their interaction is very complex.

Protein mediators are the kinins, the cytokines, such as tumour necrosis factor (TNF) and the interleukins, and the complement system. These are blood borne agents having a generalised whole body effect. The kinins are of particular interest because, in rats infused with endotoxin, the death rate was reduced significantly when they were inhibited. The study of kinins in MSOF has been limited, partly by the difficulty of assaying them. Further investigation is clearly necessary to elucidate their involvement in MSOF.

Cytokines, especially TNF, are of great current scientific interest. This thesis tries to show that, at the present time, their clinical importance is exaggerated. When measured in the blood of patients with MSOF they show a daily pulse, rising to a peak after several hours and falling back to baseline values. Concentrations of TNF during the peaks is similar to that shown in human volunteers injected with low doses of endotoxin.

Endotoxin causes the release of TNF from monocytes, which causes the symptoms of endotoxemia. But many patients with MSOF either are not infected with exogenous micro-organisms or have Gram-positive or viral infections that do not produce endotoxin. In these patients TNF has not been shown to be present. TNF antibodies can prevent all of the symptoms of endotoxemia when given before an endotoxin infusion; or some of the symptoms when given 2 hours before infusion. But when TNF antibodies are given later than this, they do not have a protective effect. TNF appears to be an agent that initiates the inflammatory response in Gram negative infection, but has a reduced involvement as the process continues. Interleukin 1 is not crucial in the development of MSOF.

The complement system is the third protein mediator of the inflammatory response involved in MSOF, especially the fragment C5a. This complement fragment stimulates neutrophil activity and is important in the leucostasis of MSOF. There is no correlation between the occurrence or severity of MSOF and increases in C5a concentration. Although C5a can cause leucostasis when administered alone, it does not cause degranulation or tissue damage. Reduction of the complement component of the blood does not reduce pulmonary damage. Complement activation, although an important component of severe inflammatory cascade activation is not the cause.

Section 2 is concerned with the lipid mediators of severe inflammatory activation. These agents have a local effect at the site of production. They are not stored, and are formed by the lipid membrane of a cell as needed. They are platelet activating factor, the cyclo-oxygenases, prostacylin and thromboxane, and the lipoxygenases, leukotrienes. The important enzyme in controlling the production of all these compounds is phospholipase.

Serum phospholipase activity measured in patients with ARDS and sepsis is raised. But it is unlikely that its measurement can be used as an index of the development and severity of ARDS. This enzyme is membrane

bound and acts locally to control the formation of the lipid mediators. Consequently its serum concentration depends on the amount that leaks away from the tissue in which it is formed. The serum concentration will be higher when vasodilation has occurred and lower in vasoconstriction. Inhibitors of phospholipase could be important in controlling the production of lipid mediators. Unfortunately most phospholipase inhibitors tend to be toxic, or steroidal, and have little effect when given 2 hours after the initiation of the inflammatory cascade. A non-toxic inhibitor of PLA may be very important in the treatment of MSOF, and further research needs to be undertaken.

The lipid mediators are each responsible for certain symptoms of MSOF and may be a mechanism by which other mediators control the development of the condition. But their production is so interactive that the production of one mediator affects the formation of others. Blockade of cyclo-oxygenases could increase the production of lipooxygenases. It seems highly unlikely that pharmaceutical intervention to reduce the activity of these mediators will improve the prognosis for patients with MSOF.

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